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Mapping the path to excellence: Evaluation of the diagnostic and treatment tools for invasive fungal infections in the balkans

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ABSTRACT

Background: In the Balkans, rising concerns about invasive fungal infections over the past decade stem from various factors. Primarily, there has been a notable uptick in immunocompromised individuals, including those with chronic illnesses like immunological and hematological diseases. Thus, it is essential to assess the region's laboratory capabilities and the availability of antifungals. This evaluation is vital for gauging the preparedness to diagnose and treat fungal infections effectively, thus minimizing their public health impact.
Methods: Data were collected via an online questionnaire targeting healthcare professionals specializing in relevant fields across diverse healthcare settings in Balkan countries. The survey covered various aspects, including diagnostic methods, imaging techniques, and available antifungal armamentarium.
Results: Responses were obtained from 50 institutions across the Balkans. While conventional diagnostic methods like microscopy (96 %) and culture (100 %) diagnostics were widely available, access to newer diagnostic tools such as molecular assays (61 %) were limited, often relying on outsourced services. Imaging modalities like ultrasound (100 %) and CT scans (93 %) were universally accessible. A variety of antifungal drugs were available, including amphotericin B formulations (80 %), echinocandins (79 %), and triazoles (100 %). However, access to newer agents like posaconazole (62 %) and isavuconazole (45 %) was inconsistent. Therapeutic drug monitoring (53 %) services were also limited.
Conclusion: The study underscores the need for equitable access to diagnostic facilities and antifungal treatments across healthcare settings in the Balkan geographic region. Improving access to molecular diagnostic tools and essential antifungal drugs, as well as implementing therapeutic drug monitoring, would optimize the management of fungal infections in the region.

Introduction

Invasive fungal infections (IFI) pose significant challenges to global healthcare systems, particularly affecting morbidity and mortality rates among immunocompromised individuals [1]. The Balkans, with their diverse socio-economic landscapes and varying healthcare infrastructures, encounter distinct hurdles in managing IFI [2–5]. Political discrepancies, with some countries part of the European Union and others not, accentuate disparities in healthcare access and resource allocation, influencing the availability of diagnostic and treatment tools [5,6].

Candida, *Aspergillus*, and *Cryptococcus* are among the fungi causing most IFI [7], ranging from bloodstream infections to invasive pulmonary syndromes, predominantly impacting populations undergoing major surgery, chemotherapy, organ transplantation, patients with autoimmune diseases treated with corticosteroids, or living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) [1–4,8,9]. Furthermore, the general population's life expectancy has increased, leading to a higher proportion of older individuals with immunosenescence [10]. Moreover, the emergence of novel challenges such as coronavirus diseases 2019 (COVID-19)-associated mycoses has exacerbated the burden of IFI [9,11–14], alongside the importance of considering other respiratory pathogens like influenza and influenza-associated pulmonary aspergillosis [11,15]. Additionally, uncontrolled use of antibiotics [16], the rise of multidrug-resistant fungal pathogens[17] and endemic fungi complicates infection management [18, especially with increasing travel among Balkan residents, heightening the risk of imported endemic mycoses and underscoring the need to enhance the region's preparedness in diagnosis and treatment.

Amidst these challenges, clinical suspicion and proper utilization of diagnostic and treatment tools are paramount in diagnosis [19–25]. Thus, investing in medical staff education, diagnostics, and treatment modalities becomes imperative. Against this knowledge gap, this manuscript aims to offer data of real situation of access to diagnostic and treatment tools in the Balkans. By examining epidemiological contexts, healthcare infrastructures, antifungal agent availability, and existing challenges, we aim to identify opportunities for improvement and advocate for targeted interventions to meet the unmet needs of patients affected by these debilitating infections.

Methods

Data collected aimed at comprehensively assessing the management capacity of healthcare professionals in dealing with IFI. Employing an online questionnaire accessible through the link www.clinicalsurveys.net/uc/IFI_management_capacity. A pre-established survey was used, which has been utilized in similar experiences, based on the globally available diagnostic and treatment tools (Supplementary Table 1). The study specifically targeted professionals specializing in clinical microbiology, haematology, infectious diseases, internal medicine, intensive medicine, and oncology, as these are the main medical specialities managing patients with IFI. Targeted professionals were reached out through professional networks, direct contacts, and by appointing a local coordinator at each participating institution, selecting medical centers based on their basic capacity and prior experience in managing IFI patients. In each country, we tailored the selection process to the local healthcare context, balancing urban and rural hospitals where feasible. This approach aimed to ensure comprehensive, reliable data collection while accounting for differences in service quality and quantity across the selected hospitals.

Diverse healthcare settings across the Balkan region were strategically chosen for participant recruitment, including hospitals, research institutions, and private laboratories spread across countries such as Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Moldova, Montenegro, North Macedonia, Serbia, Slovenia, and Romania.

The survey, designed to cover a broad spectrum of relevant topics, navigated through relevant areas such as expert perceptions of IFIs and available treatment options, the availability of diagnostic methods such as microscopy, culture, serology, antigen detection, molecular testing, and therapeutic drug monitoring (TDM), as well as imaging methods. The answers regarding treatment modalities and diagnostic methods were given dichotomously declaring if it was available or not, and if available whether it is available onsite or in other partner institution. Every answer was checked and validated before the analysis to guarantee data correctness and completeness. Only one answer was permitted per each participating institution.

Following the comprehensive data collection process, the amassed information underwent rigorous descriptive statistical analysis to distil key findings. Frequencies and percentages were computed to provide a quantitative overview of responses to various

Table 1
Baseline characteristics of participating institutions in the Balkan countries.

Microbiology laboratory		
Onsite	47/50	94.0 %
Outsourced	2/50	4.0 %
Mycological diagnosis performance		
Onsite	25/50	50.0 %
Onsite - Outsourced	21/50	42.0 %
Outsourced	3/50	6.0 %
IFI incidence perception		
Very low	17/50	34.0 %
Low	22/50	44.0 %
Moderate	9/50	18.0 %
High	2/50	4.0 %
Very high	0/50	0.0 %
Most important pathogen(s)		
Aspergillus spp.	37/50	74.0 %
Candida spp.	48/50	96.0 %
Cryptococcus spp.	23/50	46.0 %
Fusarium spp.	13/50	26.0 %
Histoplasma spp.	2/50	4.0 %
Lomentospora/Scedosporium spp.	1/16	6.3 %
Mucorales	12/50	24.0 %
Phaeohyphomycetes	1/16	6.3 %

ICU, intensive care unit; IFI, invasive fungal infection; spp., species
Full country data are available in Supplementary table 3 (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, and Greece) and in Supplementary table 4 (Moldova, Montenegro, North Macedonia, Romania, Serbia, and Slovenia)

survey items. The Statistical Package for the Social Sciences (SPSS) version 27.0, developed by IBM Corp. and headquartered in Chicago, IL, USA, served as the trusted software for data processing and analysis, ensuring the reliability and accuracy of the study outcomes.

Results

From November 2021 to June 2023, responses were gathered from 50 institutions in the Balkans overseeing IFI management in different type of patients at risk (Supplementary table 2). The distribution of sites across countries during this period was as follows: Romania 11 sites (22.0 %), Greece ten sites (20.0 %), and Bulgaria, Croatia, and Serbia each six sites (12.0 %), Slovenia four sites (8.0 %), and Bosnia and Herzegovina three sites (6.0 %). Additionally, Albania, Moldova, Montenegro, and North Macedonia each contributed responses from one site (2.0 %). Among these, only three institutions (3/50, 4.0 %) lacked an onsite microbiology laboratory, one each in North Macedonia, Romania, and Serbia. Regarding mycological diagnoses, all institutions had access, except one site in Romania, to either onsite (25/50, 50.0 %), hybrid onsite-outsourced (21/50, 42.0 %), or fully outsourced (3/50, 6.0 %) IFI diagnosis performance. None of the sites considered the incidence of IFI in their institutions as very high, but two regarded it as high, in Bulgaria and Serbia (2/50, 2.0 % each), both managing high-risk patients (Table 1).

All institutions considered *Candida* spp. as the most concerning fungal species, except for two (48/50, 96.0 %), one each in Montenegro and Serbia. Overall, *Aspergillus* spp. were the second most concerning species (37/50, 74.0 %), followed by *Cryptococcus* spp. (23/50, 46.0 %) (Fig. 1).

Microscopy access for histopathological diagnostics was widely reported as available (45/47, 95.7 %), except in one laboratory in Greece and another in Serbia. China/India ink (32/38, 84.2 %) and Giemsa stain (34/41, 82.9 %) were the most widely available microscopic methodologies, followed by potassium hydroxide (26/38, 68.4 %). Only Bulgaria, Croatia, Greece, Romania, and Serbia reported

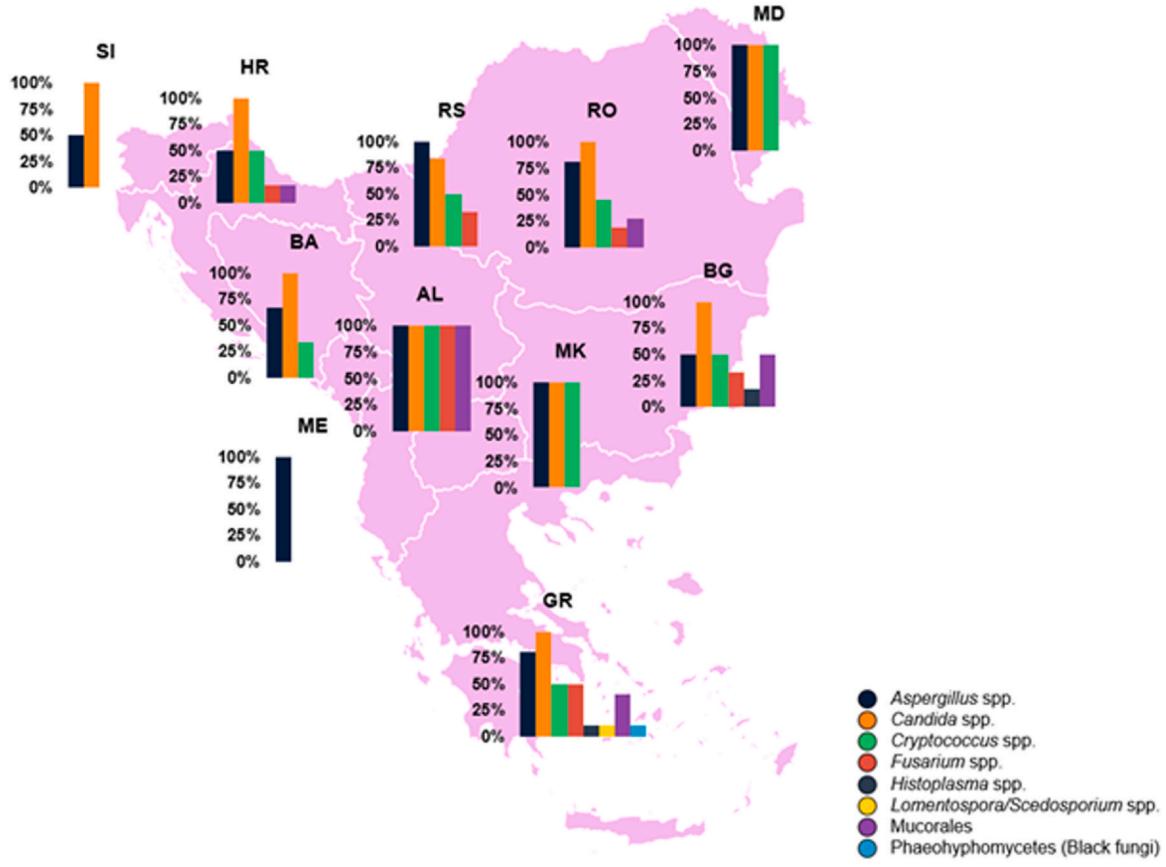

Fig. 1. Ranking of fungi perceived as those of greatest concern in the Balkan countries. **AL**, Albania; **BA**, Bosnia and Herzegovina; **BG**, Bulgaria; **GR**, Greece; **HR**, Croatia; **ME**, Montenegro; **MD**, Moldova; **MK**, North Macedonia; **RS**, Serbia; **RO**, Romania; **SI**, Slovenia. Full country data are available in Supplementary table 1 (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, and Greece) and in Supplementary table 2 (Moldova, Montenegro, North Macedonia, Romania, Serbia, and Slovenia).

Table 2
Available methodologies for fungal species identification and for diagnosis of IFI in the Balkan countries.

Access to laboratory procedures		
Microscopy	45/47	95.7 %
Methodologies		
Calcofluor white	12/32	37.5 %
Giemsa stain	34/41	82.9 %
China/India ink	32/38	84.2 %
Potassium hydroxide	26/38	68.4 %
Silver stain	15/38	39.5 %
Access to fluorescence	19/45	42.2 %
Direct examination if cryptococcosis suspected	35/45	77.8 %
Silver stain if pneumocystosis suspected	14/45	31.1 %
Direct microscopy if mucormycosis suspected	16/42	38.1 %
Culture	45/45	100.0 %
Fungal culture methods		
Agar Niger	5/34	14.7 %
Chromogen	25/31	80.6 %
Lactrimel agar	3/33	9.1 %
Potato dextrose agar	14/34	41.2 %
Sabouraud dextrose agar	40/41	97.6 %
Sabouraud dextrose agar + Chloramphenicol	26/36	72.2 %
Sabouraud dextrose agar + Gentamicin	19/36	52.8 %
Selective agar	20/33	60.6 %
Available tests for specific identification	45/45	100.0 %
Automated identification	38/41	92.7 %
Biochemical tests	28/41	68.3 %
DNA sequencing	9/42	21.4 %
MALDI-TOF	19/42	45.2 %
Mounting medium	7/36	19.4 %
Antifungal susceptibility testing	37/38	97.4 %
CLSI	7/32	21.9 %
EUCAST	12/30	40.0 %
Gradient strip test	23/31	74.2 %
Semiautomated antifungal susceptibility testing system	30/36	83.3 %
Antibody detection	32/42	76.2 %
Aspergillus spp.	31/42	73.8 %
Onsite	19/42	45.2 %
Outsourced	12/42	28.6 %
Candida spp.	26/42	61.9 %
Onsite	14/41	34.1 %
Outsourced	11/41	26.8 %
Histoplasma spp.	12/40	30.0 %
Onsite	3/40	7.5 %
Outsourced	9/40	22.5 %
Antigen detection	38/43	88.4 %
Aspergillus overall	37/43	86.0 %
Aspergillus galactomannan (ELISA)	34/43	79.1 %
Onsite	18/43	41.9 %
Outsourced	16/43	37.2 %
Aspergillus galactomannan (LFA)	22/41	53.7 %
Onsite	7/41	17.1 %
Outsourced	15/41	36.6 %
Aspergillus galactomannan (LFD)	19/42	45.2 %
Onsite	9/42	21.4 %
Outsourced	10/42	23.8 %
Candida antigen	26/43	60.5 %
Onsite	13/43	30.2 %
Outsourced	13/43	30.2 %
Cryptococcus overall	29/39	74.4 %
Cryptococcus (LAT)	25/38	65.8 %
Onsite	15/38	39.5 %
Outsourced	10/38	26.3 %
Cryptococcus (LFA)	19/39	48.7 %
Onsite	8/39	20.5 %
Outsourced	11/39	28.2 %
Histoplasma antigen	11/37	29.7 %
Onsite	2/37	5.4 %
Outsourced	9/37	24.3 %
Beta-d-glucan	24/41	58.5 %
Onsite	9/41	22.0 %
Outsourced	15/41	36.6 %
Molecular tests	25/41	61.0 %
Aspergillus PCR	22/40	55.0 %
Onsite	14/40	35.0 %
Outsourced	8/40	20.0 %
Candida PCR	19/41	46.3 %
Onsite	11/41	26.8 %
Outsourced	8/41	19.5 %
Pneumocystis PCR	19/40	47.5 %
Onsite	11/40	27.5 %
Outsourced	8/40	20.0 %
Mucorales PCR	12/35	34.3 %
Onsite	6/35	17.1 %
Outsourced	6/35	17.1 %
Access to imaging procedures		
CT	13/14	92.9 %
PET CT	3/14	21.4 %
MRI	12/14	85.7 %
PET MRI	0/14	0.0 %
Ultrasound	14/14	100.0 %
X ray	11/14	78.6 %
Bronchoscopy	12/14	85.7 %
Colonoscopy	11/14	78.6 %
Gastroscopy	11/14	78.6 %
Laryngoscopy	12/14	85.7 %
Nasal endoscopy	12/14	85.7 %

CLSI, Clinical and Laboratory Standards Institute; CT, computed tomography; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; EUCAST, European Committee on Antimicrobial Susceptibility Testing; LAT, latex agglutination test; LFA, lateral flow assay; LFD, lateral flow device; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-light mass spectrometry; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PET, positron emission tomography; spp., species
Full country data are available in Supplementary table 3 (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, and Greece) and in Supplementary table 4 (Moldova, Montenegro, North Macedonia, Romania, Serbia, and Slovenia)

global access to all of the microscopical methods in at least one local laboratory. All analysed institutions confirmed the ability to perform culture-based diagnosis in their laboratories (45/45, 100.0 %), with Sabouraud dextrose agar (40/41, 97.6 %) and chromogen (25/31, 80.6 %) being the most common fungal culture methods. Access to agar Niger (5/34, 14.7 %) and lactrimel agar (3/33, 9.1 %) was residual and possible only in Bulgaria, Romania, and Moldavia for the former, and Bulgaria and Greece for the latter. Fungal species identification was possible in all institutions (45/45, 100.0 %), mainly via automated identification (38/41, 92.7 %) and biochemical tests (28/41, 68.3 %). In parallel, 37/38 (97.4 %) institutions could perform antifungal susceptibility test analyses, mainly with semiautomated antifungal susceptibility testing systems (30/36, 83.3 %) and gradient strip tests (22/31, 71.0 %) (Table 2, Fig. 2).

Fungal antibody detection was possible in Bosnia and Herzegovina (2/3, 66.7 %), Bulgaria (5/5, 100.0 %), Croatia (4/6, 66.7 %), Greece (8/10, 80.0 %), Moldova (1/1, 100.0 %), Montenegro (1/1, 100.0 %), Romania (5/8, 62.5 %), Serbia (4/4, 100.0 %), and Slovenia (2/3, 66.7 %). Antigen test kit access was distributed similarly between countries, although no information regarding these tests was possible to retrieve from Albania or North Macedonia. Kits for *Aspergillus* spp. (37/43, 86.0 %), mainly enzyme-linked immunosorbent assays (ELISA) (34/43, 79.1 %), and *Cryptococcus* spp. (29/39, 74.4 %) were the most frequent. No access to *Candida* mannan tests was reported from Albania, Bosnia and Herzegovina, or Moldova, and β-D-glucan tests were not available in Albania and Moldova. Almost one-third of the institutions (25/41, 61.0 %) had access to at least one PCR test for fungal diagnosis, mainly for *Aspergillus* spp. (22/40, 55.0 %) and *Pneumocystis* spp. (19/40, 47.5 %). Overall, 14/14 (100.0 %) institutions had access to ultrasound, 13/14 (92.9 %) to CT, and 12/14 (85.7 %) to MRI or at least one type of endoscopy (Table 2, Fig. 2).

Regarding treatment, almost all sites could perform IFI-related surgeries (9/13, 69.2 %). In terms of drug-based treatment, at least one formulation of amphotericin B was present in 78.7 % (37/47) of institutions, especially the liposomal formulation (33/44, 75.0 %).

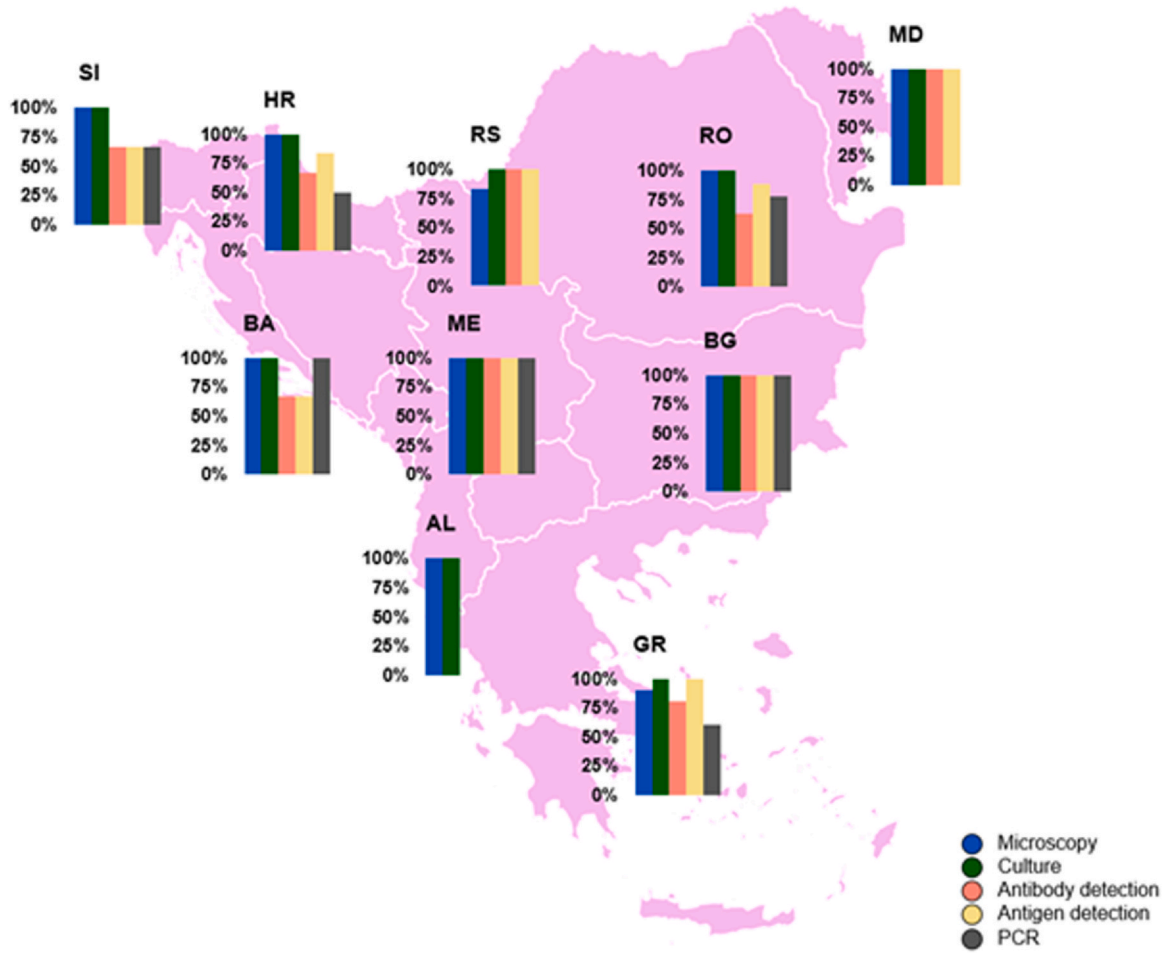

Fig. 2. Distribution of available diagnostic tests for invasive fungal infections in the Balkan countries. **AL**, Albania; **BA**, Bosnia and Herzegovina; **BG**, Bulgaria; **GR**, Greece; **HR**, Croatia; **ME**, Montenegro; **MD**, Moldova; **MK**, North Macedonia; **RS**, Serbia; **RO**, Romania; **SI**, Slovenia. Full country data are available in Supplementary table 1 (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, and Greece) and in Supplementary table 2 (Moldova, Montenegro, North Macedonia, Romania, Serbia, and Slovenia).

Additionally, at least one echinocandin was available in 78.0 % (39/50) of institutions, mainly caspofungin (38/49, 77.6 %). All institutions but one could use at least one triazole (50/51, 98.0 %), in any case fluconazole (50/51, 98.0 %). Among the mould-active triazoles, voriconazole (44/49, 89.8 %) and itraconazole (40/46, 87.0 %) were the most common ones. Flucytosine could not be administered in Slovenia, and terbinafine not in Serbia. TDM was reported in 52.6 % (20/38) of analysed sites, mainly for voriconazole (21/38, 55.3 %) (Table 3).

Discussion

Across the Balkans, laboratory procedures revealed widespread availability of microscopy and culture diagnostics, with variations observed in access to specific methodologies, so as newer technologies like antigen and antibody detection and molecular tests. Imaging procedures, such as ultrasound, CT, and MRI, were universally accessible. Additionally, a diverse range of antifungal drugs, including amphotericin B formulations, echinocandins, and triazoles, were available across institutions, with potential disparities in access to newer agents or therapeutic monitoring services.

The widespread presence of onsite microbiology laboratories in the analysed countries indicates a solid foundation for diagnosing IFIs in the region. However, the existence of fully outsourced diagnostics in some institutions suggests potential resource constraints or a strategic reliance on external expertise, usually causing diagnostic delays in acute disease. This underscores the importance of ensuring fair access to diagnostic facilities in all healthcare settings, thereby guaranteeing prompt and precise management of fungal infections.

While the majority of institutions did not consider the incidence of IFIs as very high, the recognition of elevated incidence in Bulgaria and Serbia raises concerns. This is slightly different than other published results from Europe and Asia/Pacific where more than a third assessed the incidence as moderate [6,26–29]. Moreover, these findings emphasize the importance of diagnostic capability and of surveillance systems to monitor trends in fungal infections and identify regions or populations at higher risk. Targeted interventions, such as infection control measures or antifungal stewardship programs, may be warranted to mitigate the burden of IFIs in these areas.

The universal recognition of *Candida* as the most concerning fungal species reflects their prominence in healthcare-associated infections. The acknowledgment of *Aspergillus* spp. and *Cryptococcus* spp. highlights the diverse spectrum of fungal pathogens and the need for comprehensive diagnostic and therapeutic strategies. These pathogens are marked as critical and high priority group by WHO fungal priority pathogens list as well [7], and the answers are in line with previously published data from Europe in general or Balkan neighbouring country Hungary [6,26]. Additionally, these pathogens are the most common causes of IFI not only worldwide [1], but also in this region [2–4].

Microscopy was available at almost all participating centres. The majority of responders reported access to China/India Ink and

Table 3
Available antifungal drugs and therapeutic monitoring for clinical management in the Balkan countries.

Surgery	9/13	69.2 %
Available antifungals		
Amphotericin B	37/46	80.4 %
Amphotericin B deoxycholate	19/39	48.7 %
Amphotericin B lipid complex	17/37	45.9 %
Amphotericin B liposomal	33/43	76.7 %
Echinocandins	38/48	79.2 %
Anidulafungin	27/42	64.3 %
Caspofungin	37/47	78.7 %
Micafungin	27/44	61.4 %
Triazoles	48/48	100.0 %
Fluconazole	48/49	98.0 %
Isavuconazole	17/38	44.7 %
Itraconazole	40/44	90.9 %
Posaconazole	26/42	61.9 %
Voriconazole	43/47	91.5 %
Flucytosine	14/38	36.8 %
Terbinafine	17/40	42.5 %
Therapeutic drug monitoring	20/38	52.6 %
Flucytosine	9/36	25.0 %
Onsite	6/36	16.7 %
Outsourced	3/36	8.3 %
Isavuconazole	4/13	30.8 %
Onsite	2/13	15.4 %
Outsourced	2/13	15.4 %
Itraconazole	14/36	38.9 %
Onsite	10/36	27.8 %
Outsourced	4/36	11.1 %
Posaconazole	13/35	37.1 %
Onsite	10/35	28.6 %
Outsourced	3/35	8.6 %
Voriconazole	21/38	55.3 %
Onsite	18/38	47.4 %
Outsourced	3/38	7.9 %

Full country data are available in Supplementary table 3 (Albania, Bosnia and Herzegovina, Bulgaria, Croatia, and Greece) and in Supplementary table 4 (Moldova, Montenegro, North Macedonia, Romania, Serbia, and Slovenia)

Giemsa stain. China/India ink is still the primary diagnostic tool for *Cryptococcus* in cerebrospinal fluid, especially because of its availability and turnaround time. However, its sensitivity remains low. Unlike these two methodologies, fluorescent dyes like calcofluor white, which are recommended for the diagnosis of *Aspergillus* and Mucorales infections [21,25], are used only in one-third of participating centres. Similar to China/India ink, calcofluor white method is also marked by its rapid turnaround time and extensive versatility. Nevertheless, due to its expense, it appears to predominantly serve as a privilege in high-income countries [30].

All centres reported the possibility to identify fungal species by culture. Automated identification, biochemical tests, and MALDI-TOF MS were reported as the most available methods, mostly in tertiary care institutions. MALDI-TOF MS is less available in Balkan countries than overall in Europe probably because of its initial high cost, despite this method is proven to be reliable, rapid, and cost-effective [31].

Furthermore, it becomes apparent that while conventional diagnostic modalities such as microscopy and culture diagnostics were pervasive, the accessibility of newer diagnostic tools, such as antigen tests, serologies, and molecular assays, exhibited notable inter-institutional variability. Noteworthy findings include the considerable prevalence of antibody detection and antigen tests for various fungal pathogens, particularly *Aspergillus* galactomannan and *Candida* mannan, with a significant proportion of centres favouring onsite testing for these markers. However, the adoption of molecular diagnostics, exemplified by PCR assays targeting *Aspergillus*, *Candida*, *Pneumocystis*, and Mucorales, was less widespread, often relying on outsourced services. This disparity accentuates the imperative for augmenting access to molecular diagnostic capabilities throughout the region, as these assays afford rapid and precise pathogen identification, thereby facilitating prompt and tailored therapeutic interventions. Augmenting accessibility to modern diagnostic technologies holds promise for advancing the early detection and management of IFIs, thereby fostering enhanced clinical outcomes.

In the domain of imaging procedures, despite only 14/50 centres provided feedback, our investigation highlights a widespread availability of essential modalities such as CT scans and MRIs, indicating wide imaging diagnostic capabilities across participating institutions. Notably, ultrasound imaging emerged as universally accessible, underscoring its pivotal role in the evaluation of fungal infections [19,21,22,25]. While other advanced imaging techniques like PET CT and PET MRI showed lower accessibility, their significance in specific clinical contexts remains noteworthy. Moreover, endoscopic procedures for gastrointestinal tract, larynx, lungs, or nose were widely accessible, facilitating comprehensive diagnostic evaluations in IFI cases. Concerning antifungal treatment, surgical interventions were available in the majority of centres, reflecting a multifaceted approach to IFI management encompassing both medical and surgical modalities.

All participants reported access to triazoles in their centres, including to the mould-active ones. Additionally, a significant number of centres could also count on different amphotericin B formulations and on echinocandins. These data are similar to those reported in the whole of Europe or in Asia/Pacific [6,27]. Nevertheless, the availability of triazoles is not consistent. For instance, posaconazole is available only in two thirds of centres. Despite not being included in the WHO essential drug list [32], it is suggested as a prophylactic agent for patients undergoing induction chemotherapy for acute myeloid leukemia and myelodysplastic neoplasms, as well as for allogeneic stem cell transplant patients in the post-engraftment phase [23,25,33–36]. Even less accessible is isavuconazole (in less than half of the centres) which is listed with voriconazole as a preferred first-line option for pulmonary invasive aspergillosis[25] and as salvage treatment for mucormycosis [21]. Flucytosine, which is listed as a WHO essential drug list [32], is available only in one third of centres. It is recommended in combination with amphotericin B for the treatment of cryptococcal meningitis, which is still a worrisome complication in people living with HIV and increasingly prevalent in other populations with considerable mortality prevalent in the region [37,38]. Therapeutic drug monitoring is recommended for most of azoles and for flucytosine because of variable pharmacokinetics and different drug-drug interactions [37,39]. However, TDM is only available in half of surveyed centres. Strategies to improve access to essential antifungal drugs and enhance TDM capabilities could optimize treatment efficacy and minimize the risk of adverse events including mortality, particularly in vulnerable patient populations.

The current study has several limitations. First, it is noteworthy that the number of responders per country is not proportional to the country's population. This could be explained by the lack of local contacts and by different health system organization. However, all included territories are very uniform, so it could be presumed that the diagnostic and therapeutic capacities, especially within the same countries, are homogeneous and that the presented data are representative. Still, the study's findings may have limited generalizability to populations with different demographics, healthcare settings, or regions. It is crucial to consider the sample characteristics and study context when applying these findings to other populations. Additionally, the self-reported nature of the data may introduce bias or inaccuracies, as we were unable to validate its accuracy, but only data completeness and coherence. Furthermore, data concerning turnaround time of the tests, especially when they are not done within the institution, were not taken into analysis and this aspect could significantly affect timely diagnosis as well. Finally, despite the diagnostic mycology capacity and access to antifungal

treatment in Eastern and South-Eastern Europe have been previously addressed [40], the countries covered in this earlier survey extended beyond the Balkan region, the number of Balkan institutions was more reduced than in the current work, and notably, Albania, Bosnia and Herzegovina, Bulgaria, Moldova, Montenegro, and North Macedonia were not represented.

In conclusion, it is evident that mycology laboratories in Balkan centres are generally well-equipped, although there is a notable gap in the availability of molecular diagnostic tools, which are accessible in fewer than half of the surveyed centres. The most pressing need for enhancement lies in improving the availability of essential therapeutics, particularly antifungals like flucytosine. Additionally, there is a clear opportunity to integrate therapeutic drug monitoring more extensively into clinical practice, as this approach can effectively address resistance issues and enhance patient outcomes.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interest

The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2024.102493.

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