

The Current State of Clinical Mycology in Eastern and South-Eastern Europe

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Abstract

The ability of medical centres in Eastern and South-Eastern Europe to diagnose and treat fungal infections remains unknown. In order to investigate that, here we conducted a cross-sectional online survey, released at both The International Society for Human & Animal Mycology (ISHAM) and European Confederation of Medical Mycology (ECMM) websites. A total of 31 institutions responded to the questionnaire. Most centres (87.1%, n=27) had access to *Aspergillus* spp. ELISA galactomannan testing as well as to *Cryptococcus* spp. antigen testing (83.9%, n=26). Serological tests were mostly available for *Aspergillus* species (80.6%, n=25); and most institutions reported access to mould-active antifungal drugs (83.9%; n=26), but 5-flucytosine was available to only 29% (n=9) of the participant centres. In conclusion, this study represents the first attempt to document the strengths and limitations of the Eastern and South-Eastern European region for diagnosing and treating fungal diseases. Key words: fungal infection; mycology; diagnosis; laboratory; antifungal agents.

Lay summary

Our article is about the availability of diagnostic and treatments tools related to fungal infections in the countries of Eastern and South-Eastern region. Surveys like these are important to understand the gaps and point towards the fungal infections as a global health issue.

Fungal infections still pose a challenge, due to combination of factors including poor awareness of health care workers, limited availability of diagnostic tools, treatment-related toxicities and limited access to antifungal drugs. The epidemiology of fungal infections has been described in many countries around the globe including Eastern and South-Eastern Europe,¹⁻⁶ including epidemiology of rare mold and rare yeast infections,⁷⁻⁹ but knowledge on the availability of antifungal drugs and diagnostic tools in Clinical Mycology in the region remains poorly studied. Moreover, inequities within Europe may result in differences in terms of access to medicines and diagnostic capacity in medical mycology.¹⁰

In order to fill this gap of information, we developed a cross-sectional online survey (www.clinicalsurveys.net, Questback GmbH, Cologne, Germany) that included 29 questions covering different topics in the field of clinical mycology. The survey remained open from June 2019 to May 2020 and was released online at the International Society of Human and Animal Mycology (ISHAM) and the European Confederation of Medical Mycology (ECMM) websites. Institutions were classified according to whether the laboratories potentially met the ECMM criteria for Blue Status, the initial category of the Excellence Centre Initiative (<https://www.ecmm.info/ecmm-excellence-centers>), which are: (i) the ability to identify relevant yeasts and moulds; (ii) performance of susceptibility testing on yeasts and moulds according to standard procedures; (iii) performance of *Aspergillus* antigen (galactomannan) test; (iv) availability of cryptococcal antigen testing.¹¹ This did not configure an ECMM accreditation, but rather suggested possible candidates for Blue Status, if there was an application from these institutions. Croatia already has an accredited ECMM Excellence Centre Silver since 2018, University Hospital Centre Zagreb Department of Clinical and Molecular Microbiology, and this centre did not answer to this survey. The ECMM accreditation process is part of a project which aims to provide expert consultation free of

charge in difficult-to-treat invasive fungal infections clinical cases (ECMM Expert Consultation Service).¹¹

We received 31 answers, from eleven different countries (Figure 1), including Greece (n=9), Croatia (n=5), Russia (n=5), Estonia (n=3), Serbia (n=2), Slovakia (n=2), Czech Republic (n=1), Hungary (n=1), Lithuania (n=1), Romania (n=1) and Slovenia (n=1). Amongst responders, only Russia and Serbia are not part of the European Union. The survey was answered by laboratory professionals (n=12), academics (n=8), attending physicians (n=3), infectious diseases specialists (n=2), institution directors (n=2) and other professionals who did not fit any of these categories (n=3). One responder did not inform their position. Amongst responders, 74.2% (n=23) were university hospitals or national institutes of research, 19.4% (n=6) were public hospitals, 6.5% (n=2) were oncology clinics, 3.2% (n=1) were private hospitals and one was an independent laboratory (provides diagnosis to health institutions but does not perform treatment). Multiple selection was allowed in this item.

Regarding performance of microscopy, potassium hydroxide was available for 71.0% (n=22) of institutions, India/China ink for 64.5% (n=20), Giemsa stain for 54.8% (n=17), silver stain for 16.1% (n=7) and calcofluor white for 38.7% (n=12). Fluorescent dyes in general were available for 61.3% (n=19). Automated blood culture monitoring was available for 80.6% (n=26) of institutions.

For fungal species identification, automated identification by VITEK or other commercial methods was available for 67.7% (n=21) of institutions; biochemical tests (classic mycology) were available for 54.0% (n=17), Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-ToF) for 48.4% (n=15), 32.3% used mounting medium (n=12), and DNA sequencing was accessible for 29.0% (n=9).

Susceptibility testing was available for both yeasts and moulds in 67.7% (n=21) of institutions and for yeasts only in 22.6% (n=7). Institutions used E-test strips (77.4%, n=24),

VITEK (41.9%, n=13), and broth microdilution, following the Clinical and Laboratory Standards Institute (CLSI) (35.5%, n=11) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards (41.9%, n=13).

Regarding the availability of serological testing, it was mostly available for *Aspergillus* spp. (80.6%, n=25). This was followed by *Candida* spp. (64.5%, n=20), *Histoplasma* spp. (16.1%, n=5) and *Paracoccidioides* spp. (9.7%, n=3).

Most institutions (87.1%, n=27) had access to *Aspergillus* spp. ELISA galactomannan testing, and 12.9% (n=4) had access to the *Aspergillus* spp. lateral flow assay (LFA). *Cryptococcus* spp. latex testing was available for 83.8% (n=26) of responders; *Cryptococcus* spp. LFA for 3.2% (n=1), *Histoplasma* spp. antigen detection for 12.9% (n=4), 1,3- β -Glucan for 51.6% (n=16) and *Candida* spp. antigen detection for 54.8% (n=17) of institutions.

The availability of molecular tests in Eastern and South-Eastern Europe is described in Table 1, and the availability of antifungal drugs is summarized on Table 2

Therapeutic drug monitoring (TDM) was locally available for itraconazole in 19.4% (n=6) of institutions, for posaconazole in 32.3% (n=10), for voriconazole in 29% (n=9), and for 5-flucytosine in 6.5% (n=2).

Regarding ECMM requirements for Blue Status, 48.4% (n=15) of institutions potentially fulfilled the minimum laboratory requirements. These centres were located in Russia (n=5), Greece (n=4), Croatia (n=1), Czech Republic (n=1), Hungary (n=1), Serbia (n=1), Slovakia (n=1), Slovenia (n=1).

Considering that antifungal resistance is a growing global health problem,¹² participant institutions were not ready to properly face this challenge, once they reported low access to diagnostic tools, including TDM. The most accessible TDM was to posaconazole, and that was available at only 32.3% (n=10) of institutions. The limited availability of molecular tests showed in our survey can also pose a problem, resulting in delayed diagnoses.

For instance, 5-flucytosine, considered essential by the World Health Organization, was available to only 29.0% (n=9) of participant centres, which is alarming considering the high mortality of HIV-associated cryptococcal meningoencephalitis.¹³ Although limited, the region has better availability of 5-flucytosine than other regions of the globe, such as Latin America and Africa (18% and 27%, respectively). At the same time, amphotericin B was available in any of its formulations in 83.9% (n=26) of institutions in our survey, and in 72% in Latin America and 52.5% in Africa.^{14,15}

Continuous work is necessary in order to reduce health inequities within the European continent and beyond, guaranteeing access to healthcare services in its three dimensions: coverage, affordability and availability of care. According to the World Health Organization, HIV/AIDS is more prevalent in Eastern countries when compared to other European subregions, as well as respiratory underlying conditions, considered important risk factor for fungal infections. For example, of the 136,449 people diagnosed with HIV/AIDS in Europe in 2019, 79.0% were diagnosed in the East (n=107,842).¹⁶ In some countries, efforts have been made to document the burden of fungal diseases. One example is Hungary, in which the number of difficult to treat and potentially life-threatening mycoses was estimated as at least 33,000 annually.³ Although Europe is not a main area of endemic mycoses, migration, travel, and increase of immunocompromised population with the advance of oncologic therapies, for example, maintain the burden of invasive fungal diseases.¹⁷

To tackle this problem, an important step would be making relevant fungal infections (such as azole-resistant *Aspergillus spp.* and *Candida auris* infections) notifiable diseases, building more active surveillance systems, monitoring and avoiding possible breakthrough infections.¹⁸ Furthermore, there is a need to improve infection control practices, considering that health-care-associated invasive fungal diseases are also an important cause of morbimortality. Educating health workers facing invasive fungal diseases according to the

best evidence available is also an important strategy. Initiatives such as ECMM Expert Consult provide tools to help capacitating and empowering institutions worldwide.¹¹ Improving access to rapid diagnostic methods, such as molecular tests (which were available for only half of institutions), would also strengthen infection control practices in the region.

This survey has some limitations, including the small number of responders. Once the survey was released online through ISHAM and ECMM websites, our data is also restricted to institutions which were aware of these societies. However, this is the first attempt to document the strengths and limitations of the region, regarding the capacity of diagnosis and treatment of fungal infections. Future studies are needed to compare diagnostic capabilities between different regions of Europe to identify areas of highest need. Surveys like these are important to understand the gaps and point towards the fungal infections as a global health issue, identifying the necessity of multidisciplinary actions from stakeholders and policy makers.

Conflict of interest

ACP has received research grants, given paid talks and consulted for Pfizer, Gilead, MSD, United Medical, Teva, and IMMY, not related to this study. DRF has received research support, payment for lectures and consulting fees from United Medical, Gilead, Astellas, MSD, IMMY and Pfizer, not related to this work.

In the last 5 years, DF has received payment for research grants, lectures, advisory boards, and/or travel reimbursements, not related to this study, from Pfizer, GSK, Gilead, MSD and United Medical.

MH has received grants or contracts from NIH, MSD, Gilead, Astellas and Pfizer, not related to this study. MH is also president of the ECMM, unpaid position.

OAC reports grants or contracts from Amplyx, Basilea, BMBF, Cidara, DZIF, EU-DG RTD (101037867), F2G, Gilead, Matinas, MedPace, MSD, Mundipharma, Octapharma, Pfizer, Scynexis; Consulting fees from Amplyx, Biocon, Biosys, Cidara, Da Volterra, Gilead, Matinas, MedPace, Menarini, Molecular Partners, MSG-ERC, Noxxon, Octapharma, PSI, Scynexis, Seres; Honoraria for lectures from Abbott, Al-Jazeera Pharmaceuticals, Astellas, Grupo Biotoscana/United Medical/Knight, Hikma, MedScape, MedUpdate, Merck/MSD, Mylan, Pfizer; Payment for expert testimony from Cidara; Participation on a Data Safety Monitoring Board or Advisory Board from Actelion, Allecra, Cidara, Entasis, IQVIA, Janssen, MedPace, Paratek, PSI, Shionogi; A patent at the German Patent and Trade Mark Office (DE 10 2021 113 007.7); Other interests from DGHO, DGI, ECMM, ISHAM, MSG-ERC, Wiley.

JPG has received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Gilead and Pfizer, not related to this study.

DES reports grants or contracts from Abbvie, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Dr. Reddy's, Montavit, Noventure, Nutricia, and Reckitt Benckiser, support for attending meetings from Nestle, not related to this study.

JRP reports advisory panel, consultation or research grants with Pfizer, Scynexis, Cidara, Matinas, Appili, F2G, Astellas, and Minnetronix.

CD, AC, JFM, VAA, ES, MJE, and TM have no conflict of interest to declare.

Author's Contributions

CD Writing – Original Draft, Visualization; DRF Writing – Original Draft, Visualization; MH Conceptualization, Methodology, Writing – Review & Editing; OAC Conceptualization,

Methodology, Writing – Review & Editing; AC Conceptualization, Methodology, Writing – Review & Editing; JPG Writing – Review & Editing; ES Writing – Review & Editing; MJE Writing – Review & Editing; TM Writing – Review & Editing; JFM Writing – Review & Editing; JRP Writing – Review & Editing; VAA Writing – Review & Editing; MM Writing – Review & Editing; DES Writing – Review and Editing; ACP Writing – Original Draft, Review & Editing, Supervision.

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References

1. Chrdle A, Mallátová N, Vašáková M, Haber J, Denning DW. Burden of serious fungal infections in the Czech Republic. *Mycoses*. 2015;58:6-14. doi:10.1111/myc.12384
2. Klimko N, Kozlova Y, Khostelidi S, et al. The burden of serious fungal diseases in Russia. *Mycoses*. 2015;58(July):58-62. doi:10.1111/myc.12388
3. Sinkó J, Sulyok M, Denning DW. Burden of serious fungal diseases in Hungary. *Mycoses*. 2015;58:29-33. doi:10.1111/myc.12385
4. Arsenijević VA, Denning DW. Estimated burden of serious fungal diseases in Serbia. *Journal of Fungi*. 2018;4(3):1-13. doi:10.3390/jof4030076
5. Osmanov A, Denning DW. Burden of serious fungal infections in Ukraine. *Mycoses*. 2015;58:94-100. doi:10.1111/myc.12409
6. Mareş M, Moroti-Constantinescu VR, Denning DW. The burden of fungal diseases in Romania. *Journal of Fungi*. 2018;4(1):1-7. doi:10.3390/jof4010031
7. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *The Lancet Infectious Diseases*. 2019;19(12):e405-e421. doi:10.1016/S1473-3099(19)30312-3
8. Hoenigl M, Salmanton-García J, Walsh TJ, et al. Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology. *The Lancet Infectious diseases*. 2021;21(8):e246-e257. doi:10.1016/S1473-3099(20)30784-2
9. Chen SCA, Perfect J, Colombo AL, et al. Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM. *The Lancet Infectious Diseases*. 2021;21(12):e375-e386. doi:10.1016/S1473-3099(21)00203-6
10. Scholz N. *Addressing Health Inequalities in the European Union.*; 2020. doi:10.2861/567478
11. Koehler P, Denis B, Denning DW, et al. European confederation of medical mycology expert consult—An ECMM excellence center initiative. *Mycoses*. 2020;63(6):566-572. doi:10.1111/myc.13076
12. Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. *The Lancet Infectious Diseases*. 2017;17(12):e383-e392. doi:10.1016/S1473-3099(17)30316-X

13. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *The Lancet Infectious Diseases*. 2017;17(8):873-881. doi:10.1016/S1473-3099(17)30243-8
14. Driemeyer C, Falci DR, Oladele RO, et al. The current state of clinical mycology in Africa: a European Confederation of Medical Mycology and International Society for Human and Animal Mycology survey. *The Lancet Microbe*. 2022;5247(21):1-7. doi:10.1016/s2666-5247(21)00190-7
15. Falci DR, Pasqualotto AC. Clinical mycology in Latin America and the Caribbean: A snapshot of diagnostic and therapeutic capabilities. *Mycoses*. 2019;(January):368-373. doi:10.1111/myc.12890
16. World Health Organization EC for DP and C. *HIV/AIDS Surveillance in Europe*. Vol 5.; 2020. doi:10.2900/073965
17. Benedict K, Richardson M, Vallabhaneni S, Jackson BR, Chiller T. Emerging issues, challenges, and changing epidemiology of fungal disease outbreaks. *The Lancet Infectious Diseases*. 2017;17(12):e403-e411. doi:10.1016/S1473-3099(17)30443-7
18. Ider BE, Adams J, Morton A, Whitby M, Clements A. Infection control systems in transition: The challenges for post-Soviet Bloc countries. *Journal of Hospital Infection*. 2012;80(4):277-287. doi:10.1016/j.jhin.2012.01.012
19. OMS. World Health Organization Model List of Essential Medicines. *Mental and Holistic Health: Some International Perspectives*. 2019;21:119-134. <https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?ua=1>

Table 1. Molecular tests availability according to the fungal pathogen.

	Molecular tests availability at the institution	Molecular tests performed at outsourced laboratories	Total
<i>Aspergillus</i> spp.	29.9% (n=9)	16.1% (n=5)	45.2% (n=14)
<i>Candida</i> spp.	41.9% (n=13)	9.7% (n=3)	51.6% (n=16)
<i>Pneumocystis</i> spp.	48.4% (n=15)	3.2% (n=1)	51.6% (n=16)
Other fungi	22.6% (n=7)	6.5% (n=2)	29.0% (n=9)

Table 2. Availability of antifungal drugs in Eastern and South-Eastern Europe.

Antifungal drug	Availability
Amphotericin B deoxycholate*	41.9% (n=13)
Amphotericin B lipid complex (ABLC)	35.5% (n=11)
Liposomal amphotericin B*	54.8% (n=17)
Other lipid formulations of amphotericin B	16.1% (n=5)
At least one amphotericin B formulation	83.9% (n=26)
Anidulafungin	67.7% (n=21)
Caspofungin	71.0% (n=22)
Micafungin	64.5% (n=20)
Fluconazole*	93.5% (n=29)
Itraconazole*	77.4% (n=24)
Voriconazole*	87.1% (n=27)
Posaconazole	64.5% (n=20)
Isavuconazole	22.6% (n=7)
Flucytosine*	29.0% (n=9)
Terbinafine	32.2% (n=10)

* Part of WHO Model List of Essential Medicines. For Amphotericin B, WHO considers sodium deoxycholate *or* liposomal complex as an essential medicine.¹⁹



Figure 1: Distribution of European institutions participating in this survey.

Legend: In blue, countries from institutions which answered the survey. The marker indicates countries where at least one institution potentially fulfilled the minimum requirements for ECMM Blue Status.