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ORIGINAL RESEARCH

Analysis of Antifungal Drug Resistance Among *Candida* Spp. and Other Pathogenic Yeasts Isolated from Patients in Eastern Poland: Diagnostic Problems

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Objective: The profile of *Candida* species and their sensitivity to antifungal drugs isolated from patients in Eastern Poland were analyzed. Identification and drug resistance interpretation issues for clinically significant rare species were investigated.

Methods: A total of 197 yeast isolates were analyzed. Fungal identification was conducted using biochemical tests and MALDI-TOF. Minimum inhibitory concentrations (MICs) were determined for amphotericin B, fluconazole, itraconazole, voriconazole, isavuconazole, posaconazole, caspofungin, micafungin, and anidulafungin. Interpretation of results was based on the EUCAST, CLSI recommendations, and available literature.

Results: The following species were identified: *Candida albicans* (n=78), *C. glabrata* (*Nakaseomyces glabrata*) (n=30), *C. dubliniensis* (n=23), *C. krusei* (*Pichia kudriavzevii*) (n=13), *C. parapsilosis* (n=13), *C. tropicalis* (n=7), *C. kefyr* (*Kluyveromyces marxianus*) (n=6), *C. lusitaniae* (*Clavispora lusitaniae*) (n=6), *C. lipolytica* (*Yarrowia lipolytica*) (n=3), *C. famata* (*Debaryomyces hansenii*) (n=2), *C. intermedia* (n=2), *C. guillermondii* (*Meyerozyma guilliermondii*) (n=2), *C. ciferrii* (n=1), *C. orthopsilosis* (n=1), *C. pelliculosa* (*Wickerhamomyces anomalus*) (n=1), *C. shehatae* (n=1), *C. fabianii* (*Cyberlindnera fabianii*) (n=1), *Cryptococcus humicola* (*Vanrija humicola*) (n=4), and *Saccharomyces cerevisiae* (n=3). The highest percentage of resistant strains was reported for *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis*, and *C. lusitaniae*.

Conclusion: Among the studied isolates, rare *Candida* species were identified. Their identification in routine diagnostics can be challenging, necessitating the use of MALDI-TOF MS. The wide spectrum of isolated species may complicate the establishment of a targeted antifungal therapy due to the lack of reference MIC ranges for the interpretation of antibiograms. Gradient strips are an accurate, reproducible, and convenient method for MIC determination.

Keywords: antifungal resistance, *Candida*, rare *Candida* species, antifungal susceptibility testing

Introduction

Yeasts, with the predominance of *Candida* spp., are important etiologic agents of fungal infections in humans. Out of approximately 200 species belonging to this genus, only a few are considered pathogenic to humans. Recent years have witnessed an increasing incidence of rare *Candida* species, such as *C. lipolytica*, *C. lusitaniae*, *C. famata*, *C.guillermondii*, and *C. pelliculosa*,[1–3] which present significant challenges in terms of their identification and interpretation of drug-resistance testing. The increasing availability of modern diagnostic methods (eg, MALDI-TOF, genomic

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sequencing) enables reliable species identification, providing a valuable alternative to the potential discrepancies seen with the biochemical tests of various commercial producers, and are increasingly utilized in microbiological laboratories.

Given information about the occurrence of multidrug-resistant yeasts[4,5] and the dangerous species *C. auris*,[6,7] it is important to conduct up-to-date regional analyses of the profiles of species isolated from infections and their drug resistance because they are crucial for making decisions regarding antifungal therapy. The reclassification and new nomenclature of certain *Candida* species, associated with the formation of dimorphic forms (anamorph, teleomorph), may be problematic due to previously established databases used for the interpretation of commercial identification tests. The new nomenclature of *Candida* species has been increasingly used in the literature and microbiological results, yet traditional names are still in use for easier species recognition, such as in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) recommendations.

The drug resistance profiles and information on isolates causing infections in a group of patients from a specific region of the country allow for the assessment of the current epidemiological situation in that area and should be taken into account in empirical therapy. This also includes surveillance of species with high epidemic potential, such as *C. auris*.

The aim of our study was to analyze the profile of yeast species isolated from human infections and to determine their antifungal resistance profiles. The study was conducted in accordance with currently available recommendations and data in relevant literature. A particular focus of the study was to evaluate the prevalence of multidrug-resistant yeasts and to determine whether there is a risk of *C. auris* occurrence in a region in eastern Poland (Lublin Voivodeship). We also addressed emerging issues at various stages of microbiological testing applied in routine diagnosis of yeast infections.

Materials and Methods

Article Ethics

The study was approved by the Bioethics Committee at the Medical University of Lublin (no. KE-0254/294/2020). It complies with the tenets of the Declaration of Helsinki.

Isolation and Identification of Yeasts

Clinical qualification of patients with symptoms of fungal infection focused on those at high risk due to their status and medical history. Clinical samples were collected from individual patients as part of routine hospital procedure and included: throat swabs (n=96), urine (n=19), sputum from patients with cystic fibrosis (n=17), pus from wounds (n=15), vaginal swabs (n=12), sputum (n=10), bronchoalveolar lavage (BAL) (n=6), blood (n=6), ear discharge (n=5), skin lesions (n=4), tongue swabs (n=3), discharge from the lips (n=2), discharge from a central venous catheter (n=1), and peritoneal fluid (n=1).

The samples were cultured on standard media used in microbiological diagnostics, according to routine laboratory procedures. For fungi, cultures were grown on Sabouraud Dextrose Chloramphenicol Agar (bioMérieux) at 30°C for up to 5 days. A yeast-like organism was considered the etiological agent of infection if a rich monoculture of the fungus was obtained, or if its growth was dominant over a sparse microbiota (in the case of samples from areas with microbiota, such as the throat, vagina, skin, or tongue). After confirming the presence of yeasts microscopically (Gram-stained preparation), species identification was performed using the commercial manual biochemical tests ID32 (bioMérieux) and VITEK 2YST cards (bioMérieux) in the VITEK 2com device, following the manufacturer's instructions. Final verification of the identified yeast species was conducted using the MALDI-TOF MS Biotyper system (Bruker Daltonics GmbH, Bremen, Germany), with three independent repetitions.[8]

Determination of Yeast Sensitivity to Antifungal Drugs

The minimum inhibitory concentration (MIC, mg/L) was determined using Liofilchem® MTS™ (MIC Test Strip), with a gradient of antibiotic concentrations (ranging from 0.002 to 32.0 mg/L) on the RPMI 1640 medium with 2% glucose (bioMerieux), utilizing an inoculum suspension of 0.5 McFarland in saline.[9] Incubation was conducted under aerobic conditions at 35°C. MIC values were read after 24 hours and confirmed after 48 hours. The MIC reading was performed

according to the manufacturer's recommendations for Liofilchem® MTS™ strips: for amphotericin B, the MIC value was read at the point of complete growth inhibition; for azoles, at the first point of significant growth density inhibition (80% inhibition); and similarly for echinocandins (80%). Reference strains *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *C. albicans* ATCC 90028 were used as controls to ensure the accuracy of the measurements.[9,10] Each isolate and control was tested in duplicate. For the identified species, MIC values (mg/L) were determined for amphotericin B, fluconazole, itraconazole, voriconazole, isavuconazole, posaconazole, caspofungin, micafungin, and anidulafungin. Results were interpreted according to the EUCAST and CLSI guidelines.[11–14]

Results

The analysis included yeast isolates cultured from 197 patients (107 women and 90 men) aged 1 to 98 years. Patients with cystic fibrosis, as well as hospitalized and outpatient individuals, were enrolled in the study. The selection of individuals for the study group, from which yeasts were isolated, was random and was related to the successive execution of microbiological testing orders for fungal infections in the laboratory during the period from 2022 to 2024. As a result of the conducted studies, a collection of 197 yeast strains was obtained. Species identification indicated a high level of diversity (Table 1).

Table 1 Species of Yeasts (n=197 Isolates) Cultured from Clinical Samples

Lp.	Anamorphic (Teleomorphic) Name of the Species (n=Number of Isolates)	Clinical Samples	Studied Group		
			Age Range (average)	Female (n/%)	Male (n/%)
1.	*Candida albicans* (78/39.6%)	Th[a]-44; U[b]-9; Sp[c]-5; Sm[d]-2: P[e]-6; BAL[f]-3; V[g]-3; To[h]-2; Cl[i]-1; Fp[j]-1; B[k]-1; S[l]-1	1–86 (39)	44/56	34/44
2.	*Candida glabrata* (*Nakaseomyces glabrata*) (30/15.2%)	Th-12; Sp-4; Sm-2; P-4; U-3; B-3; S-2	10–86 (62)	7/23	23/77
3.	*Candida dubliniensis* (23/11.7%)	Th-14; Sm-5; BAL-1; P-1; L[m]-1; V-1	7–91 (35)	10/43	13/57
4.	*Candida krusei* (*Pichia kudriavzevii*) (13/6.7%)	Th-5; V-3; U-2; Sp-1; Sm-1; P-1	25–90 (48)	8/62	5/38
5.	*Candida parapsilosis* (13/6.7%)	Th-5; E[n]-4; B-1; To-1; L-1; S-1	4–90 (44)	9/69	4/31
6.	*Candida tropicalis* (7/3.6%)	Th-5; U-1; V-1	4–76 (44)	4/57	3/43
7.	*Candida kefyr* (*Kluyveromyces marxianus*) (6/3.0%)	V-2; Th-1; BAL-1; P-1; U-1	54–98 (69)	5/83	1/17
8.	*Candida lusitaniae* (*Clavispora lusitaniae* (6/3.0%)	U-3; Th-1; V-1; P-1	30–60 (45)	4/67	2/33
9.	*Candida lipolytica* (*Yarrowia lipolytica*) (3/1.5%)	Th-3	6–40 (21)	3/100	0/0
10.	*Candida famata* (*Debaryomyces hansenii*) (2/1.0%)	Sm-2	20, 25	2/100	0/0
11.	*Candida intermedia* (2/1.0%)	Th-2	5, 12	1/50	1/50
12.	*Candida guillermondii* (*Meyerozyma guilliermondii*) (2/1.0%)	B-1; V-1	30, 29	2/100	0/0
13.	*Candida ciferrii* (1/0.5%)	Sm-1	22	1/100	0/0
14.	*Candida orthopsilosis* (1/0.5%)	E-1	36	0/0	1/100
15.	*Candida pelliculosa* (*Wickerhamomyces anomalus*) (1/0.5%)	Th-1	75	1/100	0/0
16.	*Candida shehatae* (1/0.5%)	BAL-1	58	0/0	1/100

(*Continued*)

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Table 1 (Continued).

Lp.	Anamorphic (Teleomorphic) Name of the Species (n=Number of Isolates)	Clinical Samples	Studied Group		
			Age Range (average)	Female (n/%)	Male (n/%)
17.	*Candida fabianii (Cyberlindnera fabianii)* (1/0.5%)	P-1	60	0/0	1/100
18.	*Cryptococcus humicola* (*Vanrija humicola*) (4/2.0%)	Sm-4	26–32 (28)	4/100	0/0
19.	*Saccharomyces cerevisiae* (3/1.5%)	Th-3	30–54 (41)	2/67	1/33

Abbreviations: Th[a], Types of clinical sample: pharyngeal swab; U[b], urine; Sp[c], sputum; Sm[d], cystic fibrosis/sputum; P[e], pus from wound; BAL[f], bronchoalveolar lavage; V[g], vaginal swab; To[h], tongue swab; Cl[i], discharge from central venous catheter; Fp[j], peritoneal fluid; B[k], blood; S[l], skin lesions; L[m], discharge from lips; E[n], ear discharge.

Nineteen species were identified (listed by the number of strains within each species): *C. albicans* (n=78), *C. glabrata* (*Nakaseomyces glabrata*) (n=30), *C. dubliniensis* (n=23), *C. krusei* (*Pichia kudriavzevii*) (n=13), *C. parapsilosis* (n=13), *C. tropicalis* (n=7), *C. kefyr* (*Kluyveromyces marxianus*) (n=6), *C. lusitaniae* (*Clavispora lusitaniae*) (n=6), *C. lipolytica* (*Yarrowia lipolytica*) (n=3), *C. famata* (*Debaryomyces hansenii*) (n=2), *C. intermedia* (n=2), *C. guillermondii* (*Meyerozyma guilliermondii*) (n=2), *C. ciferrii* (n=1), *C. orthopsilosis* (n=1), *C. pelliculosa* (*Wickerhamomyces anomalus*) (n=1), *C. shehatae* (n=1), *C. fabianii* (*Cyberlindnera fabianii*) (n=1), *Cryptococcus humicola* (*Vanrija humicola*) (n=4), and *Saccharomyces cerevisiae* (n=3). The presence of *C. auris* was not detected. For convenience, only the classical names of the species from the genera *Candida, Cryptococcus*, and *Saccharomyces* were used in subsequent elements of the work.

Based on the MIC values (mg/L), MIC_{50} and MIC_{90} were determined for the most numerous species, with isolation numbers ranging from 78 to 13 strains. For the remaining species, which included strains from 7 isolates to 1 isolate, individual MIC values were specified (Tables 2–5). Interpretation of MIC values in terms of resistance to the tested antifungal drugs was based on the EUCAST and CLSI recommendations, as well as on data in relevant literature. Table 6 presents a summary of the number (n) and percentage (%) of resistant isolates among the obtained isolates of each species, based on available sources for result interpretation, along with the recommended resistance threshold (MIC R) for each antifungal drug.

Table 2 Range of MIC (mg/L), MIC_{50} and MIC_{90} Values of the Tested Antifungal Drugs Against *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*

	***C. albicans* (n=78)**			***C. glabrata* (n=30)**		
	MIC Range	**MIC50**	**MIC90**	**MIC Range**	**MIC50**	**MIC90**
Amphotericin B	0.064–1.000	0.19	0.5	0.023–0.75	0.25	0.5
Fluconazole	0.190–≥32	0.5	0.5	0.5–32	2.0	16.00
Itraconazole	0.006–≥32	0.125	0.19	0.094-≥32	0.25	32
Voriconazole	0.006–32	0.032	0.064	0.016–8.00	0.125	3.0
Isavuconazole	0.002–≥32	0.064	0.094	0.016–1.50	0.125	0.750
Posaconazole	0.002–≥32	0.064	0.125	0.094-≥32	1.0	12.0
Caspofungin	0.006–0.500	0.19	0.19	0.064–1.00	0.25	0.75
Micafungin	0.002–0.064	0.008	0.012	0.002–0.064	0.008	0.064
Anidulafungin	0.002–0.094	0.016	0.016	0.006–0.125	0.047	0.094

(*Continued*)

Table 2 (Continued).

	***C. albicans* (n=78)**			***C. glabrata* (n=30)**		
	MIC Range	**MIC50**	**MIC90**	**MIC Range**	**MIC50**	**MIC90**
	C. dubliniensis (n=23)			***C. krusei*** (n=13)		
	MIC range	MIC50	MIC90	MIC range	MIC50	MIC90
Amphotericin B	0.008–0.5	0.064	0.25	0.002–4.0	0.5	2.0
Fluconazole	0.190–1.50	0.5	1.0	1.0-≥32	12	≥32
Itraconazole	0.016–3.0	0.125	0.5	0.125–6.0	1.5	4.0
Voriconazole	0.004–0.125	0.016	0.064	0.002–1.0	0.25	0.75
Isavuconazole	0.002–0.25	0.004	0.032	0.002–1.5	0.25	0.75
Posaconazole	0.016–0.38	0.094	0.25	0.19–1.5	0.75	1.5
Caspofungin	0.032–32	0.25	4.0	0.19–6.0	0.5	6.0
Micafungin	0.006–≥32	0.012	0.032	0.002–0.094	0.047	0.064
Anidulafungin	0.002–≥32	0.023	0.064	0.002–0.19	0.064	0.19
	C. parapsilosis (n=13)			***C. tropicalis*** (n=7)		
	MIC range	MIC50	MIC90	MIC range	MIC	
Amphotericin B	0.047–0.5	0.19	0.5	0.19–1.0	0.19; 0.19; 0.25; 0.25; 0.25; 0.75; 1.0	
Fluconazole	0.25–6,0	1.0	4.0	0.5–1.0	0.5; 0.5; 0.5; 0.75; 0.75; 1.0; 1.0	
Itraconazole	0.125–1.0	0.38	1.0	0.094–4.0	0.094; 0.125; 0.125; 0.19; 0.19; 0.25	
Voriconazole	0.016–0.38	0.064	0.19	0.032–0.125	0.032; 0.047; 0.047; 0.094; 0.094; 0.125; 0.125	
Isavuconazole	0.012–0.19	0.047	0.19	0.023–0.19	0.023; 0.032; 0.047; 0.047; 0.064; 0.125; 0.19	
Posaconazole	0.19–0.5	0.25	0.5	0.094–1.5	0.094; 0.094; 0.125; 0.125; 0.25; 0.5; 1.5	
Caspofungin	0.125–≥32	0.5	≥32	0.125–0.38	0.125; 0.125; 0.125; 0.19; 0.25; 0.25; 0.38	
Micafungin	0.25–≥32	0.75	6.0	0.008–0.064	0.008; 0.012; 0.012; 0.016; 0.016; 0.016; 0.064	
Anidulafungin	0.25–≥32	≥32	≥32	0.006–0.094	0.006; 0.008; 0.016; 0.023; 0.023; 0.032; 0.094	

Discussion

The collection of 197 yeast isolates was obtained from various clinical materials from patients with fungal infections. The collected samples were appropriate for the site of an ongoing infection. Our study was not limited to materials obtained from invasive infections but aimed to assess the spectrum of species present across a clinically diverse group of patients with infections in various locations. Biochemical identification based on commercial tests did not lead to consistent species identification in several cases when compared to repeated determinations using MALDI-TOF, as also reported by other authors. This included, for example: *C. parapsilosis* (MALDI-TOF *C. orthopsilosis*) cultured from ear discharge,[16] *C. pelliculosa* (MALDI-TOF *Cyberlindnera fabianii*) cultured from the throat,[17] *C. sivicola* (MALDI-TOF *Stephanoascus ciferrii*) from the sputum of a cystic fibrosis patient,[18] as well as *C. dubliniensis* (MALDI-TOF *Yarrowia lipolytica*) from the throat, *C. famata* (MALDI-TOF *C. intermedia*) from the throat, *C. sake* (MALDI-TOF *C. shehatae*) from BAL, *C. sake* (MALDI-TOF *C. parapsilosis*) from ear discharge, and *C. kefyr* (MALDI-TOF *C. krusei*) from an abscess.

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Table 3 Range of MIC (mg/L) and MIC Values of the Tested Antifungal Drugs for *Candida kefyr*, *Candida lusitaniae*, and *Candida lipolytica*

	***C. kefyr* (n=6)**		***C. lusitaniae* (n=6)**		***C. lipolytica* (n=3)**
	MIC Range	**MIC**	**MIC Range**	**MIC**	**MIC**
Amphotericin B	0.125–0.5	0.125; 0.125; 0.25; 0.25; 0.38; 0.5	0.003–0.25	0.003; 0.094; 0.125; 0.19; 0.25	0.064; 0.5; 0.75
Fluconazole	0.125–6.0	0.125; 0.25; 0.25; 0.38; 0.5; 6.0	0.38–6.0	0.38; 0.75; 4.0; 6.0; 6.0	0.094; 4.0; 12.0
Itraconazole	0.125–3.0	0.125; 0.19; 0.19; 0.25; 0.38; 3.0	0.094–2.0	0.094; 0.25; 0.38; 0.75; 0.75; 2.0	0.38; 3.0; 4.0
Voriconazole	0.006–0.064	0.006; 0.006; 0.012; 0.016; 0.016; 0.064	0.008–0.25	0.008; 0.016; 0.064; 0.064; 0.064; 0.25	0.047; 0.094; 0.75
Isavuconazole	0.002–0.064	0.002; 0.002; 0.003; 0.006; 0.016; 0.064	0.008–0.125	0.008; 0.012; 0.032; 0.047; 0.047; 0.125	0.064; 0.094; 0.094
Posaconazole	0.094–2.0	0.094; 0.125; 0.19; 0.25; 0.38; 2.0	0.016–0.25	0.016; 0.125; 0.125; 0.125; 0.19; 0.25	0.25; 2.0; 12.0
Caspofungin	0.125–0.38	0.125; 0.19; 0.19; 0.25; 0.38; 0.38	0.5–4.0	0.5; 0.75; 0.75; 1.0; 1.5; 4.0	1.5; 3.0; 4.0
Micafungin	0.016–0.064	0.016; 0.016; 0.023; 0.032; 0.047; 0.064	0.032–0.047	0.032; 0.032; 0.047; 0.047; 0.047; 0.047;	0.064; 0.25; 1.0
Anidulafungin	0.012–0.38	0.012; 0.023; 0.047; 0.064; 0.25; 0.38	0.047–0.25	0.047; 0.094; 0.094; 0.094; 0.125; 0.25	0.19; 0.19; 1.5

In the pool of 197 strains, eight cases of inconsistent species identification were noted, and they represented yeasts rarely isolated from clinical materials. In the case of isolation of a rare species, verification of its identification using a method other than a biochemical test should be applied. MALDI-TOF is recommended,[19] as it has been approved by the FDA, along with genetic methods. Among the other species reported in the study, their identification (confirmed by MALDI-TOF) did not present difficulties with commercial biochemical tests, and over 99% agreement in identification was observed. In recent years, access to the MALDI-TOF technology in laboratories has significantly increased and, currently, verification of bacterial and fungal species identification using this method is not problematic. Accurate identification of yeast species is crucial for the interpretation of the antifungal susceptibility profile, which is important in establishing antifungal therapy for infections in various locations and often involves specific patient groups.

Among patients included in the study, we particularly noted those with cystic fibrosis, from whom seven different species of yeast were isolated from sputum, encompassing 17 isolates: *C.* dubliniensis (n=5), *Cryptococcus humicola* (n=4), *C. albicans* (n=2), *C. famata* (n=2), *C. glabrata* (n=2), *C. ciferrii* (n=1), and *C. krusei* (n=1). Due to the use of inhaled medications, patients with cystic fibrosis are particularly susceptible to the presence of yeasts in the respiratory tract, which is associated with a more severe form of the disease.[20] The most prevalent species in the group of cystic fibrosis patients included in the study, *C. dubliniensis*, has a particular affinity with the respiratory tract in this condition, due to the hydrophobicity of its cell surface, which facilitates easier proliferation in dehydrated secretions.[21]

The recommended method for the determination of antifungal susceptibility in fungi is to establish MIC (mg/L) values. The disc diffusion method is not used; however, this method has been described and validated in some studies for selected *Candida* spp.[22] Currently, the determination of MIC values is recommended by CLSI and EUCAST. The dilution method is used, with the procedure details described by the reference centers, CLSI and EUCAST, differing slightly. This pertains to the type of wells used on the plate (round or flat), the concentration of glucose in the medium (0.2 or 2.0%), incubation time for azole testing (48 or 24 h) and *Cryptococcus* (72 or 48 h), and growth inhibition with amphotericin B (100 or 90%). Additionally, the method for reading the final result differs—CLSI recommendations suggest visual assessment, while EUCAST uses spectrometric reading.[11,12] Another method used to determine the MIC

Table 4 MIC (mL/L) Values of the Tested Antifungal Drugs for *Candida famata*, *Candida intermedia*, *Candida guilliermondii*, *Candidaciferrii*, *Candida orthopsilosis*, *Candida pelliculosa*, *Candida reliculosa*, *Candida shehatae*

	***C. famata* (n=2)**	***C. intermedia* (n=2)**	***C. guilliermondii* (n=2)**	***C. ciferrii* (n=1)**	***C. orthopsilosis* (n=1)**	***C. pelliculosa* (n=1)**	***C. reliculosa* (n=1)**	***C. shehatae* (n=1)**
	MIC	**MIC**	**MIC**	**MIC**	**MIC**	**MIC**	**MIC**	**MIC**
Amphotericin B	0.094; 0.094	0.016; 0.38	0.012; 0.016	0.75	0,5	0.125	0.125	0.38
Fluconazole	0.016; 1.5	1.0; 4.0	0.5; 1.0	>32	3.0	1.5	1.5	>32
Itraconazole	0.125; 1.0	0.125; 0.75	4.0; 8.0	0.19	1.0	0.75	0.75	0.064
Voriconazole	0.094; 0.25	0.047; 0.094	0.016; 0.032	0.25	0.19	0.004	0.064	0.75
Isavuconazole	0.125; 0.25	0.032; 0.094	0.002; 0.064	0.5	0.094	0.125	0.125	0.25
Posaconazole	0.002; 0.25	0.094; 0.125	0.75; 1.5	>32	0.5	2.0	2.0	1.5
Caspofungin	0.003; 1.5	0.19; 0.75	0.38; 2.0	0.25	>32	0.38	0.38	0.38
Micafungin	0.002; 0.19	0.016; 0.047	0.064; 0.094	0.047	6.0	0.032	0.032	0.032
Anidulafungin	0.002; 1.0	0.032; 0.032	1.5; 2.0	0.25	>32	0.016	0.016	0.38

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Table 5 MIC (mg/L) Values of the Tested Antifungal Drugs for *Cryptococcus humicola*, *Saccharomyces cerevisiae*, and *Cyberlindnera fabianii*

	***C. humicola* (n=4)**	***S. cerevisiae* (n=3)**	***C. fabianii* (n=1)**
	MIC	**MIC**	**MIC**
Amphotericin B	0.19; 0.38; 0.5; 0.75	0.094; 0.75; 1.0	0.25
Fluconazole	2.0; >32; >32; >32	6.0; 12.0; >32	0.25
Itraconazole	0.5; 1.0; 1.0; >32	2.0; 4.0; 32	0.125
Voriconazole	0.047; 0.5; >32; >32	0.047; 0.125; 1.5	0.006
Isavuconazole	0.19; 0.38; 0.38; >32	0.064; 0.125; 0.19	0.003
Posaconazole	0.5; 0.5; 12.0; >32	2.0; 3.0; 32.0	0.125
Caspofungin	0.047; 0.064; 0.094; 0.38	2.0; 4.0; 6.0	0.38
Micafungin	0.023; 0.032; 0.047, 0.064	0.094; 0.094; 0.125	0.064
Anidulafungin	0.032; 0.047; 0.064; 075	0.75; 1.0; 1.0	0.064

for fungi is a commercial method using strips impregnated with antifungal drugs, which have a scale of concentrations allowing for the reading of MIC values at the intersection of the inhibition zone. In our study, Liofilchem® MTS™ strips were used. Initially, we performed controls that confirmed the correlation of the results with the microdilution method. We determined the MICs using strips with a gradient of antifungal drug concentrations in two independent repetitions, alongside simultaneous control using reference strains, with results in each testing round conforming to recommendations.[9,10] The method using strips with a gradient of drug concentrations is simple, easy, and quick to perform and read. The ability to carry out independent, single determinations allows for broader application, such as selecting the spectrum of measured MIC values depending on fungal species and the need for specific testing in the laboratory for a particular patient. High agreement has been noted between dilution methods in broth according to the CLSI and EUCAST and tests using this method.[23] An interesting report highlights the potential use of a liquid positive blood culture in cases of candidemia for direct determination of antifungal susceptibility using strips with a gradient of drug concentrations.[24] This is somewhat analogous to performing such susceptibility tests in cases of bacteremia. When using gradient concentration strips in the laboratory, it is crucial to develop an appropriate control scheme and establish the correct categories of agreement for critical points with reference methods.[10] In our study, 19 species were identified (Table 1). The most frequently isolated species was *C. albicans*, with 78 strains accounting for 39.6% of all yeast isolates. They were cultured from 12 different types of clinical material, with the highest number cultured from the throat (44 strains). In the assessment of the culture in conjunction with clinical symptoms, a state of colonization was excluded. This localization of local candidiasis can also be a source of endogenous invasive infection. In many studies, *C. albicans* is mentioned as the dominant species in infections.[25,26] The diversity of locations may be influenced by various pathogenic possibilities and virulence factors, including the transition from yeast form to hyphal form, as well as the ability to produce biofilm and to undergo adaptation to new environmental niches. Determination of the resistance profile of the infecting yeast strain is crucial to establish an effective treatment regimen. Most *C. albicans* isolates exhibited susceptibility to the tested antifungal drugs. According to the EUCAST interpretation, the highest percentage of resistant isolates was reported for itraconazole (91%), posaconazole (79%), and anidulafungin (90%). According to the CLSI, which has higher resistance breakpoints, such a high percentage of resistant isolates was not observed.

C. glabrata was the second most numerous species. In other studies, *C. glabrata* is mentioned next,[27,28] which may be related to the geographical area from which the strains were obtained. The MIC_{90} values confirmed high resistance rates to fluconazole, posaconazole, itraconazole, and caspofungin.

Table 6 Number and Percentage (n/%) of *Candida* Isolates (the Eight Most Frequently Isolated Species) Resistant to the Tested Antifungal Drugs According to the Recommended Breakpoint MIC (MIC R) Values (EUCAST, CLSI, and Literature Data)

Lp.	Species (n)	AMB		FLU		ITC		VO		POS		CAS		MYC		AND		Reference
		MIC R	n/%	MIC R	n/%	MIC R	n/%	MIC R	n/%	MIC R	n/%	MICR	n/%	MIC R	n/%	MIC R	n/%	
1.	*C. albicans* (78)	**>1**	0/0	**>4**	7/9	**>0.06**	**71/91**	**>0.25**	8/10	**>0.06**	**62/79**	–	–	**>0.016**	13/17	**>0.03**	**70/90**	EUCAST [9–11]
		–	–	**≥8**	10/13	–	–	**≥1**	7/9	–	–	**≥1**	0/0	**≥1**	0/0	**≥1**	0/0	CLSI [9,12,15]
2.	*C. glabrata* (30)	**>1**	0/0	**≥16**	4/13	–	–	–	–	–	–	–	–	**≥0.03**	6/20	**≥0.06**	5/17	EUCAST [9–11]
		–	–	**≥64**	1/3	–	–	–	–	–	–	**≥0.5**	**13/43**	**≥0.25**	0/0	**≥0.5**	0/0	CLSI [9,12,15]
3.	*C. dubliniensis* (23)	**>1**	0/0	>4	0/0	**>0.06**	**18/78**	**>0.25**	0/0	**>0.06**	**13/57**	–	–	–	–	–	–	EUCAST [9–11]
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CLSI [9,12,15]
		>1	0/0	–	–	–	–	–	–	–	–	–	–	–	–	**>0.06**	**16/69**	[13,14]
4.	*C. krusei* (13)	**>1**	3/23	–	–	–	–	**≥0.25**	5/38	–	–	–	–	–	–	**>0.06**	3/23	EUCAST [9–11]
		–	–	–	–	–	–	**≥2**	0/0	–	–	**≥1**	**6/46**	**≥1**	0/0	**≥1**	0/0	CLSI [9,12,15]
		–	–	–	–	**>0.125**	**11/85**	–	–	–	–	–	–	–	–	–	–	[13,14]
5.	*C. parapsilosis* (13)	**>1**	0/0	**>4**	5/38	**>0.125**	**11/85**	**>0.25**	1/8	**>0.06**	**13/100**	–	–	**≥2**	2/15	**>4**	**10/77**	EUCAST [9–11]
		–	–	**≥8**	0/0	–	–	**≥1**	0/0	–	–	**≥8**	**5/38**	**≥8**	0/0	**≥8**	**9/69**	CLSI [9,12,15]
6.	*C. tropicalis* (7)	**>1**	0/0	**>4**	0/0	**>0.125**	**3/43**	**>0.25**	0/0	**>0.06**	**7/100**	**≥1**	0/0	–	–	**>0.06**	1/14	EUCAST [9–11]
		–	–	**≥8**	0/0	–	–	**≥1**	0/0	–	–	–	–	**≥1**	0/0	**≥1**	0/0	CLSI [9,12,15]

(*Continued*)

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Table 6 (Continued).

Lp.	Species (n)	AMB		FLU		ITC		VO		POS		CAS		MYC		AND		Reference
		MIC R	n/%	MIC R	n/%	MIC R	n/%	MIC R	n/%	MIC R	n/%	MICR	n/%	MIC R	n/%	MIC R	n/%	
7.	*C. kefyr* (6)	–	–	**>4**	1/17	–	–	–	–	–	–	–	–	–	–	–	–	EUCAST [9–11]
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CLSI [9,12,15]
		>1	0/0	**>2**	1/17	–	–	**>0.003**	1/17	–	–	–	–	–	–	**>0.125**	2/33	[13,14]
8.	*C. lusitaniae* (6)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	EUCAST [9–11]
		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CLSI [9,12,15]
		>1	0/0	**>2**	**4/67**	–	–	**>0.003**	**4/67**	–	–	–	–	–	–	**>0.125**	1/17	[13,14]

Notes: The grey cells in the table indicate a lack of recommendations for interpreting resistance. In the MIC R columns, the reference MIC values for resistance categories are shown (values in bold). In the column, n/% the bold and underlined values highlight a high number/high percentage of resistant isolates in the tested species group.

Abbreviations: AMB, amphotericin B; FLU, fluconazole; ITC, itraconazole; VO, voriconazole; POS, posaconazole; CAS, caspofungin; MYC, micafungin; AND, anidulafungin.

C. dubliniensis is the closest relative to *C. albicans*, which can be challenging in terms of their differentiation, which is also due to clonal characteristics. They exhibit high sensitivity to antifungal drugs.[29] In our study, among 23 strains, there were high resistance rates to itraconazole (78%) and posaconazole (57%), which may be due to the local occurrence of this resistance profile.

C. krusei isolates involved in infections show a tendency to easily acquire resistance and demonstrate resistance to fluconazole,[30] which was also confirmed by our results (MIC_{90} ≥32 mg/L). *C. krusei* strains primarily exhibited resistance to itraconazole (11 out of 13 isolates) and (MIC_{90} >32) to fluconazole and caspofungin, with six resistant isolates among all 13 isolates belonging to this species.

The occurrence of the next analyzed species, *C. parapsilosis*, varies by region in Europe. It is the second most commonly isolated species in hospitals in Southern Europe (Portugal, Spain, Italy, and Greece). This yeast is a part of the *psilosis* complex, which also includes *C.orthopsilosis* and *C. metapsilosis*.[31] In our study, *C. parapsilosis* was primarily resistant to posaconazole (all strains) and showed high resistance to itraconazole (11 out of 13 strains) and anidulafungin (10 strains). With higher resistance thresholds for anidulafungin (according to CLSI), MIC >8 mg/L, resistance was observed in 9 out of 13 isolates. In contrast, all strains of *C. tropicalis* exhibited resistance to posaconazole.

With regard to another species, *C. lusitaniae*, 3 out of 6 isolates demonstrated high MIC values for fluconazole (4.0, 6.0, 6.0) and resistance to voriconazole (4 strains). *C. lusitaniae* is a member of the normal mycobiota of animals and is rarely isolated from clinical materials. Typically, strains are sensitive to echinocandins, but there are concerns about the potential emergence of antifungal resistance in this species, for example during treatment with amphotericin B, caspofungin, and azoles.[32] Additionally, isolates of *C. lipolytica* with the MIC values of 4.0 and 12.0 were categorized as resistant to fluconazole and all were resistant to itraconazole. These MIC results can be interpreted by drawing analogies with the MIC of other species with similar values.

C. ciferrii isolated from the sputum sample of a cystic fibrosis patient posed identification challenges and showed high resistance (MIC >32) to fluconazole and posaconazole. Based on analogies with other resistant species with the MIC value of 0.5, it also exhibited resistance to posaconazole. The isolated strain of *C. orthopsilosis* was characterized by resistance to caspofungin (MIC >32), micafungin (MIC = 6.0), and anidulafungin (MIC >32). Despite its relatedness to *C. parapsilosis*, it did not show analogous resistance. The *C. shehatae* species consists of three genetically divergent subgroups that have been given varietal status: *C. shehatae* var. *shehatae*, var. *lignosa*, and var. *Insectosa*.[33] In our study, we cultured a single strain of *C. shehatae* and did not conduct a detailed genetic identification of the subspecies. It exhibited a high MIC for fluconazole (>32) and an MIC of 1.5 for posaconazole.

Among the yeasts isolated from cystic fibrosis patients, we obtained four strains of *Vanrija humicola*—previously named *Cryptococcus humicola,*[34] which exhibited high resistance to fluconazole (3 out of 4 isolates had an MIC of >32) as well as resistance at the same MIC level for voriconazole (2 strains), with one of the 4 isolates resistant to isavuconazole and posaconazole.

Three isolates of *Saccharomyces cerevisiae* cultured from the throat demonstrated high resistance to fluconazole (MIC: 6.0, 12.0, >32) and caspofungin (MIC: 2.0, 4.0, 6.0). *S. cerevisiae* is commonly referred to as "baker's yeast" and is considered a rare commensal in the digestive system. However, since the 1990s, there have been increasing reports on its involvement in infections, including invasive ones.[35] The presence of this opportunistic yeast in the throat, accompanied by symptoms of fungal infection, warrants attention, especially since the isolates in our study exhibited resistance to important antifungal drugs. Immunocompromised individuals may be particularly susceptible to infections caused by this low-virulence yeast, making it essential to include this species in microbiological studies and monitor its drug resistance.

Yeasts belonging to other species identified in the study, *including C. kefyr*, *C. famata*, *C. intermedia*, *C. guillermondii*, *C.* pelliculosa, *and C. fabianii*, did not exhibit resistance to the antifungal drugs tested.

It should be emphasized that a large proportion of the patients from whom yeast isolates were cultured were elderly or had clinically diagnosed fungal infections. The development of fungal resistance may be associated with more frequent contact with healthcare settings and a higher likelihood of colonization (presence in the microbiota) by strains originating in the hospital environment. Although our study did not include healthy patients or the analysis of their microbiota, one could hypothesize that this mechanism may be relevant for these patients. In the treatment of hospitalized patients, antifungal drugs commonly used in the empirical therapy include fluconazole and echinocandins. Overuse of these

antifungal drugs may lead to the selection of resistant isolates. Nevertheless, it is worth noting that, among the most commonly isolates species, fluconazole resistance rates were high only in *C. lusitaniae* isolates (67%), which supports its usefulness in the therapy of infections. The lowest resistance rates, observed only in *C. krusei* isolates (23%), were reported for amphotericin B. This drug is also used in the empirical therapy of many *Candida* infections.

Conclusions

Accurate species identification is fundamental to determine antifungal drug resistance. Application of MALDI-TOF technology in the study enabled to identify a diversity of yeasts, including rare species. The challenge in accurately determining drug resistance stems from the lack of interpretive criteria for MIC values in case of rarely encountered yeast species. One potential solution to interpretation difficulties is to compare values with established resistance breakpoints for species with defined MIC ranges, particularly phylogenetically-related species. We found very precise and reproducible results for MIC values using gradient concentration strips. This simple method can be successfully employed in diagnostic laboratories, allowing for rapid adaptation to the patient's situation.

The highest resistance rates were observed for itraconazole, posaconazole, and anidulafungin in the isolates representing *C. albicans*, *C. parapsilosis,* and *C. dubliniensis* species. The lowest resistance rates, observed only in *C. krusei* isolates, were reported for amphotericin B. Our study also revealed individual strains of rare species (*C. lusitaniae*, *C. lipolytica*, *C. cifferii*, *C. orthopsilosis*, *C. shehatae*, *V. humicola*, *Saccharomyces cerevisiae*) resistant to the key antifungal drugs, including fluconazole, posaconazole or caspofungin, which is a concerning phenomenon.

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Disclosure

The authors declare no conflicts of interest in this work.

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