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ORIGINAL ARTICLE

Epidemiology, clinical characteristics, outcome and biofilm forming properties in candidaemia: A single-centre retrospective 4-year analysis from Hungary

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Abstract

Background: Candidaemia is a life-threatening disease that is associated with high mortality, especially in intensive care units (ICUs). The number of comprehensive studies dealing with the epidemiologic characteristics of biofilm-related properties is limited.

Objective: This study evaluated the clinical characteristics of candidaemia, to assess the biofilm-forming properties of isolates, and to identify the risk factors of mortality.

Patients and Methods: A total of 149 candidaemia episodes from the University of Debrecen, Clinical Centre, between January 2020 and December 2023 were investigated retrospectively. The susceptibility of *Candida* isolates to fluconazole, amphotericin B, anidulafungin, caspofungin, and micafungin was evaluated and compared to the susceptibility of 1-day-old biofilms. Multivariate logistic regression analysis was applied to identify the independent predictors of 30-day mortality rate.

Results: The most common *Candida* species was *Candida albicans* (41%), followed by *C. parapsilosis* (20%), *C. glabrata* (14%), *C. tropicalis* (13%), rare *Candida* species (7%), and *C. krusei* (5%). Sixty-six percent of *Candida* isolates were biofilm formers and 44% had high metabolic activity. The 30-day mortality rate was 52%, which was higher in ICUs (65%). The logistic regression analysis revealed several factors significantly influencing mortality including ICU admission (odds ratio [OR] 2.99, 95% confidence interval [CI] 1.17–8.04, $p = 0.025$), fluconazole treatment (OR 4.12, 95% CI 1.62–11.42, $p = .004$), and pneumonia (OR 0.261, 95% CI 0.1–0.67, $p = .006$).

Conclusions: This comprehensive analysis supports the better characterisation of candidaemia in healthcare settings, which ultimately may reduce mortality among patients.

KEYWORDS
biofilm, bloodstream infections, *Candida*, Hungary, intensive care unit, mortality

1 | INTRODUCTION

Candida species are one of the main causative agents of invasive fungal infections in hospitalised patients, of which candidaemia remains the most frequently observed disease.[1] Population-based studies report that the overall pooled-incidence of candidaemia is 3.88/100,000 in Europe, from which the highest value was observed in intensive care units (ICUs) (5.5/1000 admissions).[2]

The therapeutic strategies and diagnostic opportunities for candidaemia have significantly improved in the last decade. Despite this, *Candida* bloodstream infections are associated with unacceptably high mortality rates, especially in ICUs (~40%–60%).[1,2] Although central venous catheter (CVC) management has become more effective, the usage of CVC has remained one of the most important risk factors for candidaemia, with the biofilm-forming ability of the *Candida* isolates having a pivotal role.[3–5] These sessile communities exhibit higher resistance against traditional antifungal agents compared to their planktonic counterparts, compromising the efficacy of treatment and increasing the mortality rates. Furthermore, these infected catheter lines serve as a continuous source of subsequent invasive infections.[6,7]

Based on large-scale studies, the epidemiology of candidaemia has shown a remarkable change in the past decades.[1,8–12] The ratio of *Candida albicans*-related cases have decreased dramatically from 70%–80% to 40%–60%, depending on the geographical area.[1] In parallel, the proportion of non-*albicans*-related episodes has increased significantly, and these are frequently associated with a higher resistance rate against traditional antifungal drugs.[1,8–12]

In the past three decades, the number of candidaemia-related epidemiological studies from Hungary or other east-central European countries is very limited.[13–18] In this study, our main focus was to comprehensively investigate the epidemiological data of candidaemia and the clinical outcomes in one of the largest university clinical centres of Hungary. Furthermore, we aimed to gain deep insights into the factors related to mortality and the biofilm-forming characteristics of clinical *Candida* isolates, thereby increasing our knowledge of *Candida* bloodstream infections.

2 | MATERIALS AND METHODS

2.1 | Patients and definitions

Our study was performed at a 1710-bed clinical centre at the University of Debrecen in Hungary. All patients with at least one positive haemoculture for *Candida* species between January 2020 and December 2023 were involved. In patients with more than one episode of a *Candida* bloodstream infection, exclusively the first infection was recorded for detailed analysis. Patients with multiple *Candida* species from the same candidaemia episode were excluded. Data about patient demographics, underlying medical conditions, and details of antimicrobial therapy were collected from the patients' medical history. Concurrent bacteraemia was defined as the isolation of potentially pathogenic bacteria from the same set of haemoculture samples. Outcomes of candidaemia were followed from the first positive haemoculture until 30 days after or death.

2.2 | Isolate identification and susceptibility testing of planktonic cells

Isolates were identified using matrix-assisted laser desorption ionisation/time-of-flight (MALDI/TOF) analysis as described previously.[19] After the identification, all isolates were stored in liquid bouillon with 15% glycerine at −20°C until they were tested. All stored *Candida* isolates were enriched in liquid Sabouraud dextrose agar (SDA) and plated on SDA for antifungal-susceptibility testing. The susceptibility of different isolates to fluconazole, amphotericin B, anidulafungin, caspofungin, and micafungin (all antifungals were from Merck, Budapest, Hungary) was determined using the broth microdilution method in RPMI-1640 (with L-glutamine and without bicarbonate, pH 7.0 with [3-(N-morpholino)propanesulfonic acid] buffer; Merck, Budapest, Hungary), using the Clinical and Laboratory Standards Institute (CLSI) standard M27-A3 guideline.[20]

The working concentrations of the antifungals tested were 0.06–32 mg/L, 0.015–8 mg/L, and 0.004–2 mg/L for fluconazole, amphotericin B, and the three echinocandins, respectively. A CLSI M27M44S document was used for the interpretation of the obtained minimum inhibitory concentrations (MICs).[21] The MIC values for amphotericin B were reported as wild type (<2 mg/L) or non-wild type (>2 mg/L), given the lack of defined clinical breakpoints. When a given MIC value was off-scale, one dilution higher than the highest concentration tested was used for analysis. All isolates were examined in three independent experiments and the median values were used for further analysis. Quality control was assessed using *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 in each experiment. Minimum inhibitory concentrations for planktonic cells (pMICs) were determined visually following a 24-h incubation period at 35°C. In the case of fluconazole and the tested echinocandins, partial-inhibition criterion (at least 50% growth reduction compared with the positive control) was applied, as recommended by CLSI guideline, while for amphotericin B, the total inhibition criterion (100% growth reduction compared with the positive control) was applied.[20]

2.3 | Testing of biofilm-forming ability

One-day-old biofilms were prepared as described in Pierce et al.[22] Briefly, the isolates were subcultured on SDA. *Candida* cells were harvested by centrifugation (3000 × g for 10 min) and were washed three times in sterile physiological saline. Afterwards, the pellets were resuspended in 5 mL of sterile physiological saline and the final density of the fungal suspension (1×10^6 CFU/mL) was prepared in RPMI-1640 using a Burker's chamber (Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt Germany). One-hundred microlitre of the *Candida* suspension was added to polystyrene flat-bottom

96-well microtitre plates (TPP, Trasadingen, Switzerland) and was incubated statically for 24 h at 35°C. Following the incubation period, the medium was removed and pre-formed biofilms were washed three times with sterile physiological saline.[19,22,23]

One-day-old biofilm masses were screened using a crystal violet assay. Furthermore, the metabolic activity of sessile cells was examined using an XTT (2,3-bis[2-methoxy- 4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) assay, as previously described by O'Toole and Hawser et al., respectively.[19,24,25] In the case of the crystal violet assay, a total of 125 μL of crystal violet solution (0.1%) was added to the wells containing pre-washed 1-day-old biofilms, then the wells were incubated for 15 min at room temperature. The unbound fraction of crystal violet was removed, and plates were washed three times with 200 μL of physiological saline. Afterwards, 125 μL of acetic acid solution (30%) was added to the wells to solubilise the bound fraction of crystal violet. Following a 15-min incubation period, a total of 100 μL of solution was added to a sterile 96-well plate, which was read spectrophotometrically at 540 nm. Unexposed wells contained 100 μL of 30% acetic acid.[19,23,24]

To quantify the metabolic activity of biofilms, an XTT assay was used, as described previously.[19,23,25] Briefly, XTT solution (Merck, Budapest, Hungary) (0.5 g/L) was supplemented with menadione (Merck, Budapest, Hungary) (10 mM prepared in 100% acetone) to a final concentration of 1 μM. A 100 μL aliquot of XTT/menadione solution was added to the wells containing the pre-washed 1-day-old biofilms, as well as to negative control wells. Afterwards, the plates were incubated in darkness for 2 h at 35°C, and then 80 μL of supernatant from each well was measured photometrically at 492 nm.[19,23,25]

2.4 | Cut-off determination for biofilm formation

Previously, a number of groups adopted the classic-quartile or tercile-based cut-off determination of biofilm-forming ability in the case of a given *Candida* population[19,26,27]; however, this approach may have several limitations and may show false positivity regarding biofilm formation, especially at lower optical density (OD) values.[28] Here, the cut-off value of biofilm development was determined by first plotting the ranked OD values then drawing a tendency curve in both the crystal violet assay and the XTT-assay. Then, the inflection point was obtained based on a polynomial regression, as described previously.[29] As result of this mathematical analysis, we received those values both in the case of biofilm mass and metabolic activity determination, which indicate the threshold of biofilm-forming ability.

2.5 | Antifungal-susceptibility testing of biofilms

The above-described XTT assay was used to assess the activity of five antifungal agents against 1-day-old biofilms.[19,25] The tested concentrations in the sessile MIC (sMIC) determination ranged from 1–512 mg/L, from 0.016–8 mg/L, and from 0.008 to 4 mg/L for fluconazole, amphotericin B, and the echinocandins, respectively. In the case of *C. parapsilosis*, the echinocandin concentrations tested were 2–1024 mg/L due to the well-known intrinsic *FKS* hot spot mutation(s).[30] When an MIC value was off-scale, one dilution higher or lower than the highest or lowest tested concentration was used for analysis. Regarding sMIC-value determination, 1-day-old biofilms were washed three times with 200 μL of sterile physiological saline to remove unadhered cells. A total of 100 μL of 0.5 g/L XTT in 1 μM menadione solution was added into all wells. Afterwards, plates were covered and incubated in the dark at 35°C for 2 h. Following the incubation period, 80 μL of the supernatant was removed and transferred to a new, sterile 96-well plate to measure the absorbance spectrophotometrically at 492 nm.[19,25] The MICs were defined as the lowest concentration that exerted at least a 50% metabolic activity decrease of the biofilms for all antifungal agents.[19,25] All isolates were tested in three independent biological replicates, and the median value of the three measures was used for further analysis. *C. albicans* SC5314 was used in all biofilm experiments as a quality control strain.

2.6 | Statistical analysis

The total mass and the metabolic activity of 1-day-old biofilms of *Candida* isolates were analysed using the Kruskal-Wallis test with Dunn's post-test. The differences in MICs against given antifungal drugs for planktonic and sessile cells were analysed using the Wilcoxon matched-pairs tests. Fisher's exact test was applied to investigate whether any of the risk factors in patients predisposed them to infection with biofilm-forming isolates. The effect of biofilm production, as well as the different patient characteristics, comorbidities, and 30-day mortality rates were analysed using the multivariate logistic regression model. The performance of the established logistic regression model in predicting mortality was evaluated using a receiver-operating characteristic (ROC) curve analysis, and the sensitivity, specificity, positive predictive values, and negative predictive values were calculated. GraphPad Prism 10.1.1 software was used for data analysis and graph editing, respectively. The results from statistical analysis were considered significant if the obtained $p < .05$.

2.7 | Ethical approval

The authors confirm that the ethical policies of the journal, as noted on the journal's guidelines for authors page, have been adhered to, and the appropriate ethical review committee approval has been received. This study was approved by the institutional ethics committee at the University of Debrecen, Regional and Institutional Research Ethics Committee (DE RKEB/IKEB) (permission number 5775-2021).

3 | RESULTS

A total of 149 patients with candidaemia were involved in our study. Patients' demographic and clinical characteristics, treatment and

outcomes, as well as the distribution of *Candida* species were presented in Figure 1 and Table 1. The majority of patients (86/149 [58%]) were male and the median age was 64 years (ages ranged from 0 to 93). CVC usage (88/149 [59%]), ICU admission (75/149 [50%]), total parenteral nutrition (65/149 [44%]), invasive mechanical ventilation (54/149 [36%]), and pneumonia (51/149 [34%]) were the most common risk factors for candidaemia (Table 1). *C. albicans* was the most frequently isolated *Candida* species (61/149 [41%]), followed by *C. parapsilosis* (29/149 [20%]), *C. glabrata* (21/149 [14%]), *C. tropicalis* (19/149 [13%]), and other less common species (11/149 [7%]) including *Cyberlindnera fabianii* (3/11), *Candida lusitaniae* (3/11), *Candida guilliermondii* (2/11) and *Candida metapsilosis* (2/11). One episode of *Candida dubliniensis* was seen. The lowest prevalence was observed in the case of *C. krusei* (8/149 [5%]). Overall, the 30-day mortality rate was 52% (78/149) (Table 1, Figure 1). The annual mortality rates were 48% (23/48), 53% (20/38), and 44% (14/32), for 2020, 2021, and 2022, respectively. It is noteworthy that the highest mortality rate was observed in 2023 (21/31 [68%]) (Figure 1). Regarding species-specific 30-day mortality, the highest value was observed in the case of a rare *Candida* species-related candidaemia (8/11 [73%]), followed by *C. glabrata* (13/21 [62%]), *C. albicans* (31/61 [51%]), *C. parapsilosis* (14/29 [48%]), *C. tropicalis* (9/19 [47%]) and *C. krusei* (3/8 [38%]). Regarding the administered antifungal therapy, fluconazole was the most frequently used antifungal drug (79/149 [53%]), followed by anidulafungin (22/149 [15%]), caspofungin (15/149 [10%]), and micafungin (9/149 [6%]) (Table 1). For 17 patients, antifungal-treatment status was unknown.

Due to the high ratio of the usage of CVCs (88/149 [59%]), an extensive analysis was performed in terms of the biofilm-forming ability of *Candida* isolates and their sessile-susceptibility pattern to antifungal drugs. Figure 2A,B show the biofilm mass and the metabolic activity of 1-day-old biofilms formed by different isolates of *Candida* species. Biofilm masses showed by different *Candida* isolates were evaluated using the crystal violet assay. The polynomial regression analysis showed that the corresponding OD value indicating biofilm formation was 0.057 (OD_{540nm}); therefore, the number of isolates with low and high biofilm mass was 50 and 99, respectively (Figure 2A). Similar to the crystal violet-based biofilm mass screening, sessile *Candida* isolates were categorised based on their metabolic activity using the XTT assay and polynomial regression analysis of the obtained OD values. The OD value indicating biofilms with high metabolic activity was 0.075 (OD_{492nm}); therefore, the number of isolates with low and high metabolic activity was 83 and 66, respectively (Figure 2B). *C. tropicalis* isolates exhibited significantly higher biofilm masses compared to *C. albicans*, *C. glabrata* and *C. krusei* ($p < .0001$–$.01$). Moreover, their metabolic activity was significantly higher compared to *C. albicans*, *C. glabrata* and some less-common *Candida* species ($p < .0001$–$.05$; Figure 2A,B).

The MIC values and susceptibility patterns of 149 clinical *Candida* isolates are shown in Table 2. The median pMIC values of isolates ranged from 0.06–64 mg/L, 0.03–2 mg/L, 0.004–2 mg/L, 0.008–2 mg/L, and 0.004–2 mg/L for fluconazole, amphotericin B, anidulafungin, caspofungin, and micafungin, respectively (Table 2). Based on CLSI breakpoints, the susceptible, dose-dependent susceptibility, and resistance categories of fluconazole was observed for one *C. albicans* and one *C. tropicalis* isolate, respectively. Regarding *C. glabrata*, all isolates showed dose-dependent susceptibility to fluconazole. Concerning echinocandin susceptibility, isolates resistant to caspofungin, micafungin, or anidulafungin were not found. Anidulafungin and micafungin inhibited all *C. albicans* isolates at ≤0.25 mg/L, but merely 95% were susceptible

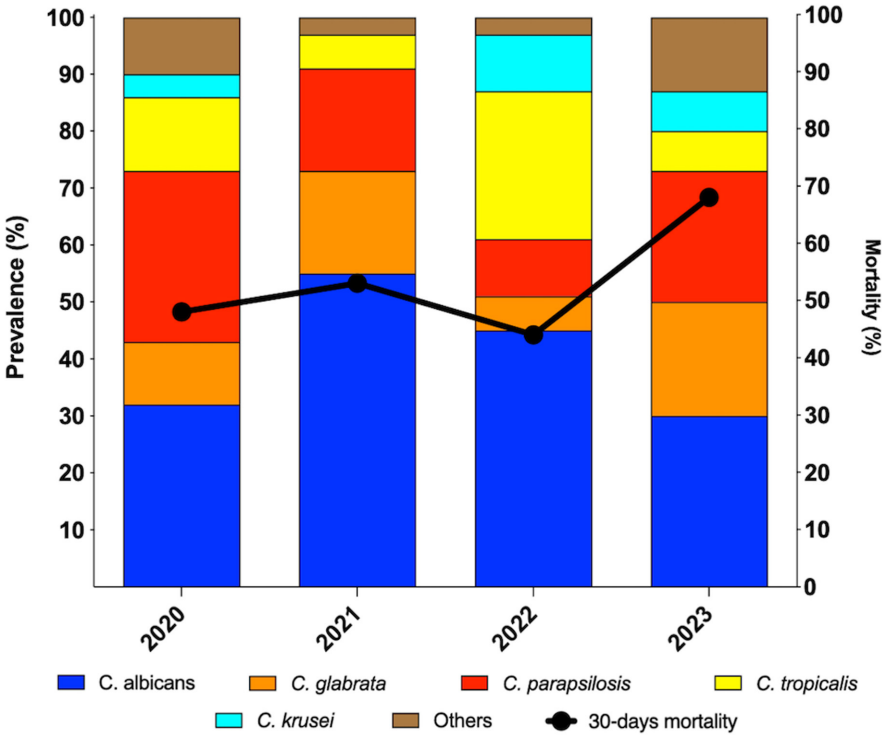

FIGURE 1 Distribution of *Candida* species and total 30-day mortality of candidaemia episodes by year.

TABLE 1 Predisposing factors, microbiological characteristics, and clinical variables for *Candida* bloodstream infection of patients with candidaemia, including comparison between subgroups according to biofilm mass and metabolic activity of biofilms. Fisher's exact test was used to investigate whether any of the risk factors in patients predisposed to infection with biofilm-former isolates.

	All ($n=149$)	No biofilm formers ($n=50$)	Biofilm formers ($n=99$)	*p*-value	Low metabolic activity ($n=83$)	High metabolic activity ($n=66$)	*p*-value
Age median (range)	64 (0–93)	66 (1–86)	63 (0–93)	.249	63 (1–93)	68.5 (0–90)	.514
Gender							
Female	63 (42%)	22 (44%)	41 (41%)	.861	33 (40%)	30 (45%)	.508
Male	86 (58%)	28 (56%)	58 (59%)	.861	50 (60%)	36 (55%)	.508
Isolated *Candida* species							
C. albicans	61 (41%)	26 (52%)	35 (36%)	.055	31 (37%)	31 (47%)	.247
C. parapsilosis	29 (20%)	8 (16%)	21 (21%)	.050	14 (17%)	15 (23%)	.409
C. glabrata	21 (14%)	9 (18%)	12 (12%)	.331	20 (24%)	1 (2%)	**.0001**
C. tropicalis	19 (13%)	0 (0%)	19 (19%)	**.0004**	1 (1%)	18 (27%)	**.0001**
C. krusei	8 (5%)	4 (8%)	4 (4%)	.443	8 (10%)	0 (0%)	**.001**
Other *Candida* spp.	11 (7%)	3 (6%)	8 (8%)	.751	9 (11%)	2 (3%)	.113
Intensive care unit	75 (50%)	25 (50%)	50 (51%)	1.000	40 (48%)	35 (53%)	.622
Hypertension	90 (60%)	33 (66%)	57 (58%)	.377	52 (63%)	38 (58%)	.614
Diabetes mellitus	39 (26%)	13 (24%)	26 (41%)	1.000	22 (27%)	17 (26%)	1.000
Chronic renal failure	53 (36%)	22 (44%)	31 (31%)	.149	33 (40%)	20 (30%)	.301
Circulatory system diseases	20 (13%)	11 (22%)	9 (9%)	**.04**	12 (16%)	8 (12%)	.810
Immunosuppression	28 (19%)	10 (20%)	18 (18%)	.826	17 (20%)	11 (17%)	.674
Invasive mechanical ventilation	54 (36%)	20 (40%)	34 (34%)	.728	32 (39%)	22 (33%)	.607
Central venous catheter	88 (59%)	28 (56%)	60 (61%)	.601	44 (53%)	44 (67%)	.097
Total parenteral nutrition	65 (44%)	25 (50%)	40 (40%)	.297	37 (45%)	28 (42%)	.868
Haematological malignancy	13 (9%)	4 (8%)	9 (9%)	1.000	8 (10%)	5 (8%)	.774
Solid malignancy	27 (18%)	12 (24%)	15 (15%)	.259	15 (18%)	12 (18%)	1.000
Abdominal surgery	26 (17%)	8 (16%)	18 (18%)	.822	11 (13%)	15 (23%)	.192
Concomitant bacteremia	40 (27%)	12 (24%)	28 (28%)	.696	19 (23%)	21 (32%)	.265
COVID-19	18 (12%)	10 (20%)	8 (8%)	.059	9 (11%)	9 (14%)	.622
Pneumonia	51 (34%)	19 (38%)	32 (32%)	.584	28 (34%)	23 (35%)	1.000
30 days mortality	78 (52%)	25 (50%)	53 (54%)	.301	42 (51%)	36 (55%)	.714
Receipt of systemic antibiotics	126 (85%)	41 (82%)	85 (86%)	.632	73 (88%)	53 (80%)	.255
Antifungal regimens for treatment							
Fluconazole	79 (53%)	18 (36%)	61 (62%)	**.005**	39 (47%)	40 (61%)	.103
Voriconazole	3 (2%)	1 (2%)	2 (2%)	1.000	2 (2%)	1 (2%)	1.000
Amphotericin B	4 (3%)	3 (6%)	1 (1%)	.110	3 (4%)	1 (2%)	.630
Caspofungin	15 (10%)	6 (12%)	9 (9%)	.576	11 (13%)	4 (6%)	.178
Micafungin	9 (6%)	2 (4%)	7 (7%)	.718	6 (7%)	3 (5%)	.732
Anidulafungin	22 (15%)	11 (22%)	11 (11%)	.09	14 (17%)	8 (12%)	.490

The results were considered significant if the $p<.05$ (bold).

to caspofungin. Three *C. albicans* isolates exhibited intermediate susceptibility. The susceptibility rates of *C. glabrata* were 100%, 87% and 52% for anidulafungin, micafungin, and caspofungin, respectively. Four and 10 *C. glabrata* isolates showed intermediate susceptibility for micafungin and caspofungin, respectively. The susceptibility rates of *C. tropicalis* were 100%, 100%, and 79% for anidulafungin, micafungin, and caspofungin, respectively. Four *C. tropicalis* isolates showed intermediate susceptibility to caspofungin. All *C. krusei* isolates were susceptible to anidulafungin and micafungin, while one isolate had intermediate susceptibility to caspofungin (Table 2).

SMIC values were determined in the biofilms produced by *C. albicans* and in non-*albicans* isolates with high metabolic activity, as determined according to the above-described polynomial regression analysis-based classification (Figure 2B). In the case of biofilms, the median sMIC values ranged from 1 to 1024 mg/L,

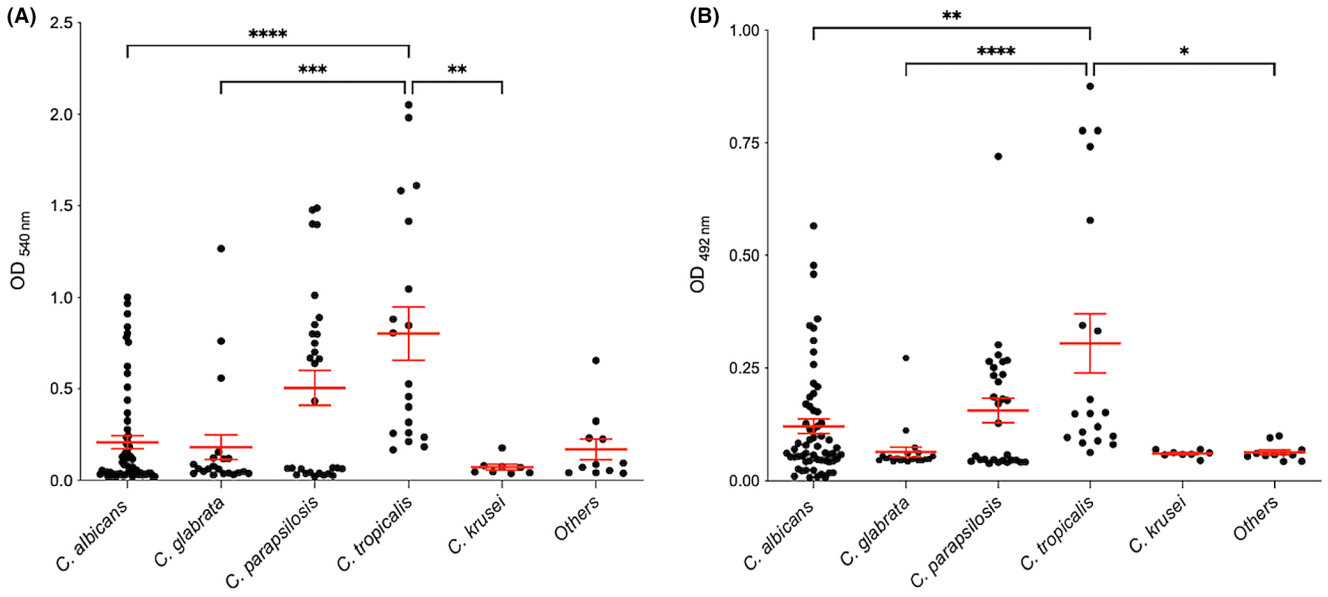

FIGURE 2 Biofilm formation of different *Candida* species by crystal violet (A) and XTT (B) assays. Biomass and metabolic activity were quantified spectrophotometrically by reading absorbance at 540 and 492 nm, respectively. Three replicates were used for each isolate with the mean of each represented. The biofilm mass and the metabolic activity of biofilms by different *Candida* species were analysed using Kruskal-Wallis test with Dunn's post-test. $^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$, respectively.

0.125–4 mg/L, 0.008–1024 mg/L, 0.016–512 mg/L, and 0.004–1024 mg/L for fluconazole, amphotericin B, anidulafungin, caspofungin, and micafungin, respectively (Figure 3A–E). SMICs to fluconazole were significantly higher in all isolated species compared to the pMIC values ($p < .001$) (Figure 3A). Regarding amphotericin B, there were no significant differences in activity against *C. glabrata* and *C. krusei* planktonic cells and biofilms (Figure 3B). Generally, echinocandins had good activity against both planktonic cells and 1-day-old biofilms. Caspofungin exhibited statistically similar activity against planktonic cells and biofilms of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and rare *Candida* species ($p > .05$) (Figure 3C). In the case of micafungin, the sMIC values were significantly higher for *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis* and *C. krusei* isolates compared to their planktonic counterparts ($p < .01$–$.001$) (Figure 3D). Anidulafungin showed lower activity against *C. albicans*, *C. parapsilosis* and *C. tropicalis* biofilms compared to planktonic susceptibility ($p < .001$) (Figure 3E).

We assessed the relationship between biofilm mass/metabolic activity and species characteristics, as well as underlying conditions. Notably, all *C. tropicalis* isolates were biofilm formers based on the crystal violet assay ($p = .0004$). In addition, 95% of *C. tropicalis* biofilms showed high metabolic activity ($p = .0001$). It is noteworthy that all *C. krusei* biofilms and 95% of *C. glabrata* isolates exhibited low metabolic activity ($p = .001$ and $.0001$, respectively) (Table 1).

In the multivariate regression analysis, we examined the factors affecting 30-day mortality in patients with candidaemia. Based on our analysis, ICU admission (odds ratio [OR]: 2.99; 95%CI: 1.17–8.04; $p = .025$) and fluconazole treatment (OR: 4.12; 95%CI: 1.62–11.42; $p = .004$) were independent predictors of mortality. In the case of pneumonia, the obtained OR was below 1. Nevertheless, the analysis was significant (OR: 0.26; 95%CI: 0.1–0.67; $p = .006$). Figure 4 presents the area under the curve in the case of our ROC analysis. The sensitivity, specificity, positive predictive value, and negative predictive value of the obtained mortality predictive model were 78.2%, 74.6%, 75.7%, and 77.2%, respectively.

4 | DISCUSSION

We performed a four-year retrospective analysis of candidaemia in one of the biggest university clinical centres in Hungary. Our main findings were that overall 30-day mortality remains high, especially in the case of uncommon *Candida* species and *C. glabrata*-related candidaemia episodes. Moreover, ICU admission and fluconazole treatment were independent predictors of 30-day mortality.

The distribution of *Candida* species among candidaemia have been extensively studied in various healthcare settings and patient populations in the past decades.[1,31–33] Our institutional epidemiology pattern was in line with the international trends regarding the ratio of candidaemia caused by *C. albicans* and non-*albicans* species. In this study, *C. parapsilosis* was the most commonly identified non-*albicans* species, followed by *C. glabrata* and *C. tropicalis*. The SENTRY surveillance program showed that the second-most frequently isolated *Candida* species was *C. glabrata*, with the exception of South America where *C. parapsilosis* was the most frequently reported non-*albicans* species.[34] In the Asia–Pacific region, *C. albicans* was found to be the most common species,

TABLE 2 Activity of antifungal agents against planktonic form of *Candida* isolates.

Species (*n*)	Drug	Planktonic MIC (mg/L)				Number of S-DD isolates number (%) of	Number of intermediate susceptibility isolates number (%) of	Number of resistant isolates number (%) of
		Range	Mode	MIC_{50}	MIC_{90}			
C. albicans (61)	FLU	0.06–1	0.125	0.125	0.25	1 (2%)	NA	0 (0%)
	AMB	0.06–0.5	0.25	0.25	0.5	NA	NA	NA
	ANI	0.004–0.125	0.008	0.008	0.015	NA	0 (0%)	0 (0%)
	CAS	0.008–0.5	0.25	0.25	0.25	NA	3 (5%)	0 (0%)
	MICA	0.004–0.25	0.008	0.008	0.03	NA	0 (0%)	0 (0%)
C. parapsilosis (29)	FLU	0.25–1	0.5	0.5	0.5	0 (0%)	NA	0 (0%)
	AMB	0.06–1	0.125	0.25	0.5	NA	NA	NA
	ANI	0.5–2	1	1	2	NA	0 (0%)	0 (0%)
	CAS	0.5–2	2	2	2	NA	0 (0%)	0 (0%)
	MICA	0.25–2	1	1	2	NA	0 (0%)	0 (0%)
C. glabrata (21)	FLU	2–8	8	8	8	21 (100%)	NA	0 (0%)
	AMB	0.25–2	0.5	0.5	1	NA	NA	NA
	ANI	0.004–0.25	0.06	0.03	0.06	NA	0 (0%)	0 (0%)
	CAS	0.125–0.25	0.125	0.125	0.25	NA	10 (48%)	0 (0%)
	MICA	0.004–0.25	0.008	0.008	0.125	NA	4 (19%)	0 (0%)
C. tropicalis (19)	FLU	0.125–64	0.25	0.25	0.5	0 (0%)	NA	1 (5%)
	AMB	0.03–0.5	0.25	0.25	0.5	NA	NA	NA
	ANI	0.008–0.06	0.03	0.03	0.03	NA	0 (0%)	0 (0%)
	CAS	0.25–0.5	0.25	0.25	0.5	NA	4 (21%)	0 (0%)
	MICA	0.008–0.5	0.06	0.06	0.125	NA	0 (0%)	0 (0%)
C. krusei (8)	FLU	16–64	64	32	64	NA	NA	NA
	AMB	0.5–2	2	2	2	NA	NA	NA
	ANI	0.03–0.125	0.06	0.06	0.125	NA	0 (0%)	0 (0%)
	CAS	0.25–0.5	0.25	0.25	0.5	NA	1 (12%)	0 (0%)
	MICA	0.125–0.25	0.25	0.25	0.25	NA	0 (0%)	0 (0%)
Others (11)[a]	FLU	0.125–8	0.5	1	4	NA	NA	NA
	AMB	0.03–0.5	0.25	0.25	0.25	NA	NA	NA
	ANI	0.03–2	0.03	0.03	0.25	NA	NA	NA
	CAS	0.06–1	1	0.25	1	NA	NA	NA
	MICA	0.06–2	0.25	0.25	1	NA	NA	NA

Abbreviations: AMB, amphotericin B; ANI, anidulafungin; CAS, caspofungin; FLU, fluconazole; MICA, micafungin; NA, not applicable; SDD, susceptible-dose dependent.

[a]Includes *Cy. fabianii* (*n* = 3), *C. lusitaniae* (*n* = 3), *C. guilliermondii* (*n* = 2), *C. metapsilosis* (*n* = 2), *C. dubliniensis* (*n* = 1).

followed by *C. tropicalis* and *C. parapsilosis*, with a prevalence of 36%, 31% and 16%, respectively.[35] In accordance with Latin-American (5%–49%) and current Hungarian (20%) data, similar *C. parapsilosis* dominance was observed in bloodstream infections in Italy (28%–41%),[36] Spain (25%),[37] and Greece (37%).[38] In the past years, several studies described a prevalence of echinocandin- or fluconazole-resistant *C. parapsilosis* isolates of more than 5% and 10%, respectively[39,40]; however, in our examination period, there were no *C. parapsilosis* isolates resistant to these agents. Rare *Candida* species, which generally account for less than 10% of candidemia, are emerging among sevearly ill patients.[41,42] Here, the proportion of candidaemia episodes caused by these uncommon *Candida* species was 7%, which was higher than the ratio of *C. krusei*-related bloodstream infections (5%). Nevertheless, we did not find any risk factors predisposing patients to these rare *Candida* species. In accordance with the study published by Tsai et al., *C. lusitaniae* was the most frequently identified rare *Candida* species in our survey (27%), together with *Cy. fabianii* (27%).[42] In a similar study, *Cy. fabianii* has emerged as a significant, uncommon yeast with the same frequency as *C. lusitaniae*.[43]

Koehler et al. (2019) performed a European epidemiologic meta-analysis focusing on the mortality rates of candidaemia.[2] Based on their findings, the overall 30-day mortality rate was 38%, which corresponded to the observed mortality in teaching hospitals. Our reported

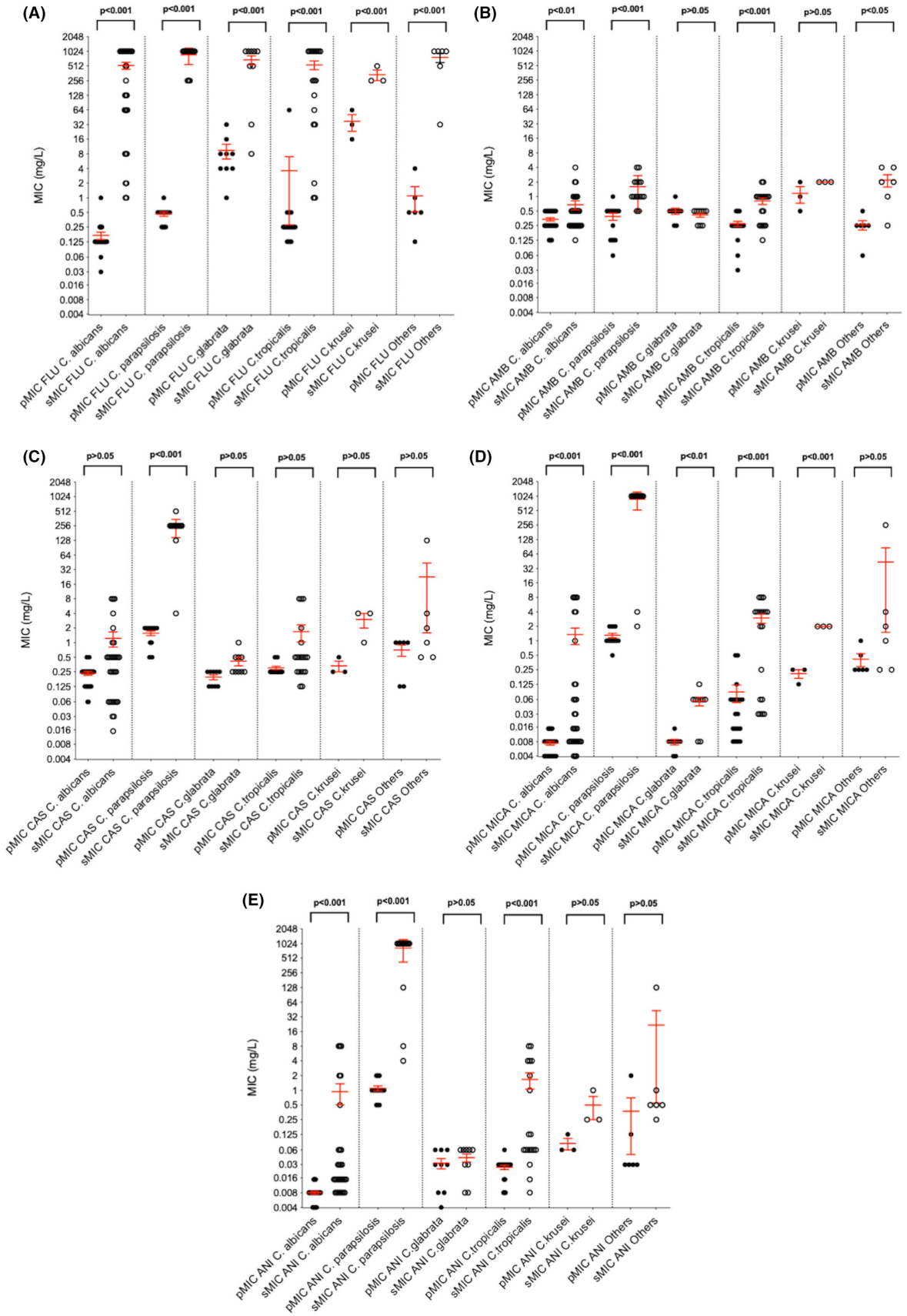

FIGURE 3 Planktonic (pMIC) and sessile MIC (sMIC) distribution of *Candida* species with high metabolic acitivtiy to fluconazole (FLU) (A), amphotericin B (AMB) (B), caspofungin (CAS) (C), micafungin (MICA) (D) and anidulafungin (ANI) (E). Three replicates were used for each isolate with the median of each represented. The results were considered significant if the $p < .05$.

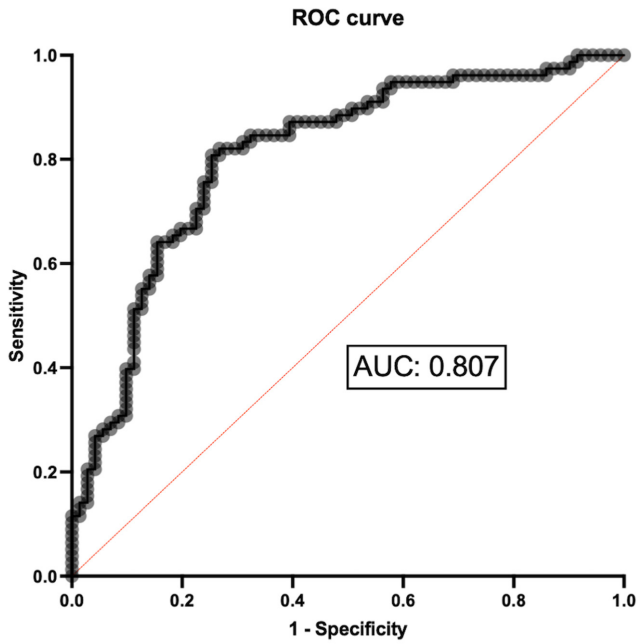

FIGURE 4 Receiver operating characteristic (ROC) curve of the model to predict mortality. AUC corresponds to area under curve.

30-day mortality rate (52%) was significantly higher compared with the findings derived from the above-mentioned meta-analysis.[2] It is noteworthy that our study revealed a significantly increased mortality rate in 2023 (68%) compared to the average of previous years (48.3%±4.5%). This phenomenon may be explained by the higher number of *C. glabrata* and uncommon *Candida* species in 2023, which were associated with higher mortality. Based on the multiple regression analysis, ICU admission was a strong independent predictor of mortality, similarly to previous studies.[44–46] Regarding our institutional ICUs, a mortality rate of 65% was observed, which is alarmingly higher than the current European ICU-based studies.[2] In one of the largest European multicentre trials on the epidemiology of ICU-acquired candidaemia, the 30-day mortality rate was 42%,[47] while a large-scale French study reported a 50% mortality rate.[48] A prospective analysis from the French REA-RAISIN network reported a mortality rate of 52% in the case of candidaemia among patients in ICU.[49]

Since CVC usage and/or total parenteral nutrition were present in the majority of candidaemia episodes of our study, the biofilm-forming ability of isolates was tested, as well as the susceptibility profile of these sessile communities. Biofilm development has been considered one of the major virulence factors of different *Candida* species, which serve as sources of persistent infection and can potentially negatively affect the function of implanted devices.[6,7]

In 2015, the European Society for Clinical Microbiology and Infectious Disease released a guideline to support healthcare professionals in the diagnosis of biofilm associated infections highlighting several clinical and laboratory procedures.[50] Different biofilm susceptibility methods were described; however, there is currently no routine test or protocol for this purpose in the clinical practice. In addition, the rationale of sessile susceptibility testing in practice in terms of positive outcome of *Candida* bloodstream infection is questionable.[51] While there are many in vitro investigations in which planktonic and sessile susceptibility toward different antimicrobial agents are compared, the number of comprehensive studies where these data are linked to the clinical outcome of therapy with these drugs is limited. There may be an overall positive correlation between planktonic and biofilm susceptibility profile; however, the decreased susceptibility in biofilms is independent of resistance in planktonic cells. In addition, the relation between planktonic and biofilm susceptibility is a drug-dependent characteristics; moreover, the given stage and maturation of biofilms tested significantly influence the obtained results.[51] Even if we could implement standardised biofilm model(s) in the routine clinical microbiology workflow, their long-term success will ultimately depend on whether using them improves the clinical outcome of a treatment.

Previous studies have described a potential correlation between biofilm formation and mortality.[19,25,52,53] Here, we did not find a correlation between biofilm formation and mortality; however, this discrepancy may be secondary to the fact that mortality in these patients is related not only to fungal sepsis, but to multiple additional risk factors. Furthermore, a threshold-calculation algorithm indicating biofilm formation may differ for each of the studies, which may distort the statistical analysis. Atiencia-Carrera et al. reported that *C. tropicalis* was the most common species with high biofilm formation (67.5%).[54] Similarly, our analysis showed the highest biofilm mass for *C. tropicalis*. Furthermore, 100% of these isolates are biofilm formers that show high metabolic activity. Although higher metabolic activity may be associated with higher mortality,[19] our *C. tropicalis* data did not support this, as the overall 30-day mortality rate for *C. tropicalis* was 47%.

Concerning treatment strategies, the most commonly administered antifungal drug was fluconazole (53%). Moreover, we reported a 31% echinocandin usage of all documented antifungal treatments. Based on a meta-analysis performed by Domingos et al., fluconazole treatment was rated the worst for overall response (17%), which coincides with the current study where fluconazole usage was an independent predictor of mortality.[55] Previous surveys compared echinocandin-based treatment with non-echinocandin-based therapy, and they concluded that echinocandins were more associated with improved clinical success than azoles.[56,57] Interestingly, in our analysis, there was no relevant difference between azole-related and echinocandin-related mortality rates (53% and 43% for echinocandin and fluconazole, respectively). Bretagne et al. published similar findings, where the replacement of antifungal first-line treatment from fluconazole to echinocandin was not associated with a decrease in total mortality, regardless of the *Candida* species involved and the antifungal-susceptibility patterns.[58] Regarding anti-biofilm activity, the lowest difference in planktonic and sessile activity of antifungals was observed in case of caspofungin; however, this positive pattern was not observed in clinical outcomes because caspofungin-related mortality rate was 53%, which did not differ significantly from the 50% and 56% mortality rates observed for anidulafungin and micafungin, respectively.

Taken together, our study presented the epidemiological pattern of candidaemia episodes in a Hungarian university clinical centre. The

overall ICU-related 30-day mortality rate was significantly higher compared to European data. Moreover, the observed mortality to uncommon *Candida* species is also alarming. Our analysis described that ICU admission and fluconazole treatment are independent predictors of mortality. Surprisingly, there was no relevant difference between azole-related and echinocandin-related mortality rates. Our comprehensive analysis supports the better characterisation of *Candida* bloodstream infections, which ultimately may diminish mortality among patients.

AUTHOR CONTRIBUTIONS

Fruzsina Kovács: Conceptualization; investigation; methodology; writing – original draft; visualization. **Noémi Balla:** Methodology; investigation. **Aliz Bozó:** Methodology; investigation. **Andrea Harmath:** Methodology; investigation. **Ágnes Jakab:** Methodology; investigation. **Zoltán Tóth:** Methodology; investigation; visualization; formal analysis. **Fruzsina Nagy:** Investigation; methodology. **László Majoros:** Data curation; writing – review and editing; formal analysis. **Renátó Kovács:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing; visualization; validation; software; project administration; supervision; resources; data curation; investigation; formal analysis; methodology.

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CONFLICT OF INTEREST STATEMENT

László Majoros received conference travel grants from Cidara, MSD, Astellas and Pfizer. All other authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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