Indian Journal of Medical Microbiology 60 (2026) 101065

Contents lists available at ScienceDirect

Indian Journal of Medical Microbiology

journal homepage: www.journals.elsevier.com/indian-journal-of-medical-microbiology

Original Research Article

Candidozyma auris: Identification, antifungal susceptibility, and caspofungin-induced paradoxical growth

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ARTICLE INFO

Keywords:
C. auris
Identification
Antifungal susceptibility
Eagle effect

ABSTRACT

Purpose: The aim of this study is to evaluate the effectiveness of VITEK®2 and CHROMagar™ *Candida* Plus agar in the identification of *C. auris*, using MALDI-TOF MS results as the reference standard. Additionally, to assess antifungal susceptibility, the study compares the results of two widely used commercial methods in routine laboratory practice: VITEK®2 and Sensititre™ YeastOne™ (SYO).

Methods: A total of 63 *C. auris* isolates were included in the study. VITEK®2 results were evaluated in comparison to SYO, and categorical agreement, major error (ME), and very major error (VME) rates were calculated. MIC50, MIC90 values and resistance rates determined by both methods were compared. The Eagle effect was also investigated.

Results: The VITEK®2 results (74 %) and the morphological characteristics of colonies grown on CHROMagar™ *Candida* Plus (100 %) were found to be consistent with MALDI-TOF MS identification. In the SYO method, the MIC values for amphotericin B, fluconazole, and voriconazole were found to be higher compared to those obtained by VITEK®2. No resistance was detected against micafungin and anidulafungin. Resistance observed in relation to caspofungin MIC values was identified as the Eagle effect. MIC50 and MIC90 values determined by VITEK®2 were lower than those obtained by SYO, except for amphotericin B, for which VITEK®2 showed a higher MIC90 value.

Conclusion: In our study, it was observed that CHROMagar™ *Candida* Plus can be used as a screening method for the identification of *C. auris* in hospitals without access to MALDI-TOF MS. Incompatibility was detected in antifungal susceptibility testing, and the rates of major error (ME) and very major error (VME) were found to be high. For caspofungin, the high dilution range in the SYO method was determined which also detected the Eagle effect.

1. IntroductionNTRODUCTION

*Candidozyma auris (*formerly *C. auris)* is an opportunistic fungal pathogen that has rapidly emerged worldwide over the past decade, causing hospital-acquired outbreaks. It was initially classified within the *Candida haemuli* (CAH) clade and poses a global health threat due to its multidrug resistance. However, given the phylogenetic distance of this clade from the type species of the *Candida* genus, phylogenomic and comparative genomic analyses were conducted. These analyses resulted in *Candida auris* and related species being reassigned to the newly established genus *Candidozyma* [1]. During the COVID-19 (Coronavirus Disease 2019) pandemic, its spread increased notably in intensive care units (ICUs) and palliative care settings in our country. *C. auris* has been listed as a critical priority fungal pathogen by the World Health Organization due to its global impact and clinical challenges. It has simultaneously emerged on five continents [2,3].

In Turkey, *C. auris* was first isolated from a clinical case by Kurt et al., in 2021 [4], and detected in our laboratory in the same year [5]. Unlike other *Candida* species, *C. auris* can grow at high temperatures (40–42 °C) and survive in hyperosmotic conditions (>10 % NaCl) [6]. It is associated with high mortality, particularly in ICU patients with multiple comorbidities [7].

C. auris is capable of colonizing human skin, resisting common disinfectants, and surviving for extended periods on healthcare equipment and in hospital environments. These characteristics facilitate clonal transmission and environmental contamination, making *C. auris* a significant public health concern. Its identification challenges, along with both intrinsic and acquired antifungal resistance, further complicate

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https://doi.org/10.1016/j.ijmmb.2026.101065
Received 6 January 2025; Received in revised form 20 January 2026; Accepted 21 January 2026
Available online 21 January 2026

infection control and treatment strategies [8]. The most common invasive infection caused by *C. auris* is candidemia [9].

Accurate and timely identification of *C. auris* is essential for effective infection control. Several automated commercial systems with updated databases can now reliably identify *C. auris* isolates. Given the multidrug-resistant nature of this pathogen, antifungal susceptibility testing (AFST) is critical for patient management. AFST results are also vital for epidemiological surveillance. Although the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method is the gold standard for AFST, it is time-consuming and operator-dependent. Therefore, many laboratories, including ours, utilize commercial kits.

Among them, the VITEK®2 system is widely used for testing *Candida* species; however, limited data exist regarding its accuracy in detecting resistance in *C. auris*, and results should be interpreted with caution [10,11]. In this study, we compared *C. auris* identification results using CHROMagar™ *Candida* Plus, the VITEK®2 yeast identification card, and MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry). For antifungal susceptibility testing, we evaluated the performance of the VITEK®2 AST-YS08 card against the Sensititre™ YeastOne™ (SYO) assay, a widely used commercial colorimetric method known for its ease of use and ready-to-use format. Identification accuracy, categorical agreement, major error (ME), very major error (VME) rates, and the presence of the caspofungin-induced paradoxical growth effect (Eagle effect) were assessed.

2. Material methods

2.1. Identification of C. auris and antifungal susceptibility testing

This study was a prospective study carried out between December 2021 and February 2024. A total of 63 *C. auris* isolates were included in the study, originating from blood (n = 40), urine (n = 17), tracheal aspirates (n = 4), and wound swabs (n = 2). Clinical samples were cultured on Sabouraud Dextrose Agar (SDA), Brain Heart Infusion (BHI) agar with 5 % sheep blood, and SDA supplemented with chloramphenicol and cycloheximide. Incubation was performed at 30–37 °C. Isolates were subsequently subcultured on SDA for further analysis.

Identification was primarily carried out using MALDI-TOF MS (BioMerieux, France)system. Additionally, 27 isolates were tested using the VITEK®2 YST card (bioMérieux, France; software version 9.03.3). To evaluate colony morphology for visual identification, all isolates were also cultured on CHROMagar™ *Candida* Plus.

The SYO assay is a colorimetric broth microdilution method. Fungal suspensions were prepared according to the manufacturer's instructions and 100 µL of the inoculum was dispensed into each drug well using an automated pipetting system. Plates were incubated at 37 °C for 24 h. The growth medium contains resazurin, a redox indicator that changes from blue (no growth) to pink (growth) when reduced by viable fungal cells. The MIC (Minimum Inhibitory Concentration) was defined as the lowest concentration that retained a blue color.

The VITEK®2 system (bioMérieux, Marcy l'Etoile, France) is a fully automated platform based on spectrophotometric detection, used for both species identification and MIC determination. A standardized fungal suspension and a card containing dried antifungal agents (amphotericin B, micafungin, caspofungin, fluconazole, and voriconazole) at 4–6 concentration levels are loaded into the system. Inoculation, incubation, growth monitoring, and result interpretation are performed automatically. An average incubation time of 15.5 h has been reported to be sufficient for agents such as amphotericin B, voriconazole, and fluconazole, while shorter incubation is adequate for echinocandins. Due to its speed and automation, VITEK®2 is widely used in clinical microbiology laboratories. Samples with identified ME and VME (described down) were subjected to duplicate repeat testing to confirm the results.

2.2. Investigation of the Eagle Effect for caspofungin

The Eagle effect was evaluated at 21 and 24 h of incubation in isolates that were resistant to caspofungin but remained susceptible to anidulafungin and micafungin. The Eagle effect was defined as occurring when fungal growth initially appeared to be inhibited at higher caspofungin concentrations (≥1 µg/mL) at 21 or 22 h of incubation on SYO plates, but full regrowth was observed in all wells by 24 h [12].

2.3. Data analysis

Statistical analysis was conducted using IBM SPSS Statistics version 22 (IBM SPSS, Turkey). In this study, the categorical agreement between antifungal susceptibility results obtained by the Sensititre™ YeastOne™ and VITEK®2 systems was evaluated. The required sample size was calculated based on the ability to detect a difference between an expected agreement rate of 90 % and a minimum acceptable agreement rate of 75 %, with 80 % statistical power and a significance level of 5 %. According to the power analysis using the normal approximation method, a minimum of 97 isolates was required. However, only 63 isolates were included in the study, and the statistical power with this sample size was calculated to be approximately 61.8 %. Therefore, the results should be interpreted with caution, considering the limitation of the sample size. In addition to descriptive statistics (frequency, percentage), Cohen's kappa (κ) analysis was performed to assess the agreement between categorical variables.

Categorical agreement (susceptible or resistant) between SYO and VITEK®2 results was evaluated based on tentative breakpoints. The accuracy of VITEK®2 in detecting resistance and susceptibility was assessed using the SYO method as the reference standard. Very major error (VME) was defined as the misclassification of a resistant isolate by VITEK®2 as susceptible, while major error (ME) referred to the misclassification of a susceptible isolate by SYO as resistant by VITEK®2.

Susceptibility interpretations (Susceptible [S] or Resistant [R]) were based on the tentative breakpoints published by the U.S. Centers for Disease Control and Prevention (CDC) for fluconazole, amphotericin B, anidulafungin, caspofungin, and micafungin. Although no breakpoint has been officially established for voriconazole, susceptibility interpretations were performed using epidemiological cutoff values (ECVs) defined in previous studies [12,13].

3. Results

Among the 27 *C. auris* isolates analyzed using the VITEK®2 system, 20 isolates (74 %) were correctly identified in agreement with MALDI-TOF MS results, while 7 isolates (26 %) were reported as "unidentified" by the VITEK®2 system.

All isolates cultured on CHROMagar™ *Candida* Plus medium exhibited characteristic light-blue colonies surrounded by a halo (Fig. 1). Thus, it can be distinguished from other *Candida* species.

Table 1 presents the MIC50, MIC90 values, and resistance rates obtained by both methods. Resistance rates to fluconazole, voriconazole, and amphotericin B were higher with the SYO method compared to VITEK®2. No resistance to voriconazole was observed using the VITEK®2 system. Although resistance to caspofungin was also higher in SYO, all isolates were reported as susceptible to micafungin by SYO. In contrast, VITEK®2 reported one isolate as resistant to micafungin. All isolates were found to be susceptible to anidulafungin, which is only included in the SYO panel. The caspofungin resistance detected by SYO prompted further investigation into the Eagle effect (paradoxical growth) (Fig. 2).

Antifungal susceptibility results obtained by Sensititre™ YeastOne™ and VITEK®2 are presented in Table 2. Taking SYO as the reference, the categorical agreement, major error (ME), and very major error (VME) rates for VITEK®2 were as follows: Fluconazole 35/63 (55.5 %)

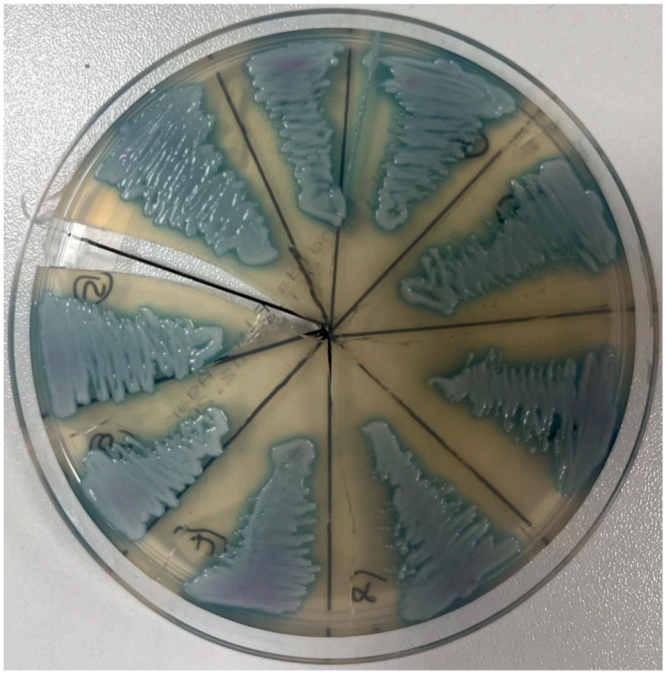

Fig. 1. *C. auris* growth image in CHROMagar™ *Candida* Plus.

agreement; 11/63 (17.4 %) ME; 17/63 (27 %) VME, Amphotericin B: 32/63 (50.8 %) agreement; 8/63 (12.7 %) ME; 23/63 (36.5 %) VME, Caspofungin: 53/63 (84 %) agreement; 1/63 (1.6 %) ME; 9/63 (14.3 %) VME, Micafungin: 62/63 (98.4 %) agreement; 1/63 (1.6 %) ME; 0/63 (0 %) VME.

For voriconazole, 23 isolates classified as resistant by SYO were identified as susceptible by VITEK®2.

In the 24-h incubation performed using the SYO method, resistance to caspofungin was observed in 9 isolates. Therefore, the Eagle effect (paradoxical growth) was investigated in these isolates.

At the 21st hour of incubation, a blue color (indicating no fungal growth) was observed at caspofungin concentrations of ≥2 μg/mL in these isolates; however, by the 24th hour of incubation, a pink color indicating fungal growth was detected in all wells. The Eagle effect for caspofungin can be detected when wide dilution ranges are tested, as in the Sensititre™ YeastOne™ method. Consequently, the 100 % VME rate observed for caspofungin in VITEK®2 was interpreted not as a true classification error, but rather as a result of this paradoxical growth phenomenon.

4. Discussion

C. auris forms light blue colonies with a distinctive blue halo on CHROMagar *Candida* Plus, enabling the exclusion of many other *Candida* species based on colony morphology. Nevertheless, it may exhibit visual similarities to *Candida albicans*, which produces green colonies, and *Candida tropicalis*, which forms blue colonies on the same medium. In the study conducted by Bentz et al., CHROMagar *Candida* Plus demonstrated high sensitivity (97 %) and specificity (100 %) for the identification of *C. auris* when tested with isolates; however, its sensitivity decreased to 60 % when directly plating skin-swab specimens

Table 1
MIC50, MIC90 values and resistance rates determined by SYO and VITEK® 2.

	Sensititre Yeast One			VITEK® 2		
ANTIFUNGAL	MIC50	MIC90	Resistance Rate(%)	MIC50	MIC90	Resistance Rate(%)
FZ	64	256	79,36	32	32	69,84
VOR	0,5	8	36,5	0,12	0,25	0
AB	2	2	63,49	1	8	39,68
CAS	0,25	8	14,28	0,12	0,25	1,58
MF	0,12	0,25	0	0,06	0,12	1,58
ANI	0,12	0,25	0	NA	NA	NA

FZ:Flucanazole, **VOR**:Voriconazole, **AB:**Amphotericin B, **CAS**:Caspofungin, **MF**:micafungin, **ANI**:Anidulafungin, Resistance Rate (%) = (Number of resistant isolates/ Total number of isolates) × 100.

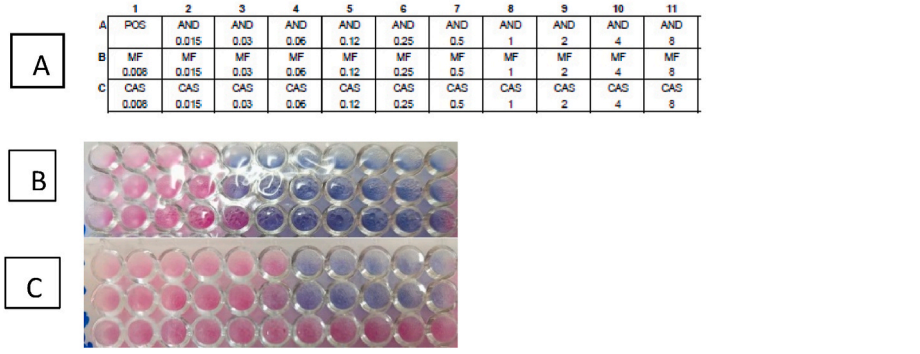

Fig. 2. Development of Eagle Effect for Caspofungin in SYO plate.

Table 2
Evaluation of antifungal susceptibility results by VITEK® 2 and SYO method.

FZ	Sensititre Yeast one												
VITEK® 2	**Dilution**	0,12	0,25	0,5	1,00	2,00	4,00	8,00	16,00	32,00	64,00	128,00	256,00	**Total**
	0,25	1	0	0	0	0	0	0	0	0	0	1	0	2
	4,00	0	0	0	0	0	0	0	0	0	0	0	1	1
	8,00	0	0	0	0	0	0	0	0	2	0	1	1	4
	16,0	1	0	0	0	0	0	0	0	1	2	4	4	12
	32,0	6	0	0	0	0	0	2	3	8	8	4	13	44
	Total	8	0	0	0	0	0	2	3	11	10	10	19	63

AB	Sensititre Yeast one							
VITEK® 2	**Dilution**	0,12	0,25	0,50	1,0	2,0	4,0	8,00	**Total**
	0,25	0	0	2	2	8	0	2	14
	0,50	0	0	1	5	6	1	1	14
	1,00	0	0	3	2	5	0	0	10
	2,00	0	2	1	3	4	0	1	11
	4,00	0	0	0	0	4	0	0	4
	8,00	0	0	0	2	7	0	1	10
	Total	0	2	7	14	34	1	5	63

VOR	Sensititre Yeast one											
VITEK® 2	**Dilution**	0,008	0,015	0,03	0,06	0,12	0,25	0,5	1,0	2,0	4,0	8,0	Total
	0,12	4	1	1	2	10	7	8	1	0	0	21	55
	0,25	0	0	0	0	0	2	1	0	0	0	1	4
	0,50	1	0	0	1	0	0	1	0	0	1	0	4
	1	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0	0
	Total	5	1	1	3	10	9	10	1	0	1	22	63

CAS	Sensititre Yeast one												
VITEK® 2	**Dilution**	0,008	0,015	0,03	0,06	0,12	0,25	0,50	1,00	2,00	4,00	8,00	**Total**
	0,12	3	1	2	4	3	8	4	0	0	0	8	33
	0,25	4	3	1	1	6	9	2	1	0	0	1	28
	0,5	0	0	0	0	0	1	0	0	0	0	0	1
	8	0	0	0	0	0	0	1	0	0	0	0	1
	Total	7	4	3	5	9	18	7	1	0	0	9	63

MF	Sensititre Yeast one											
VITEK® 2	**Dilution**	0,008	0,015	0,03	0,06	0,12	0,25	0,50	1	2	4	8	**Total**
	0,06	0	1	3	8	22	11	2	0	0	0	0	47
	0,12	0	0	0	1	8	4	1	0	0	0	0	14
	0,50	0	0	0	0	0	1	0	0	0	0	0	1
	1	0	0	0	0	0	0	0	0	0	0	0	0
	4,00	0	0	0	0	0	1	0	0	0	0	0	1
	Total	0	1	3	9	30	17	3	0	0	0	0	63

FZ:Flucanazole, **VOR**:Voriconazole, **AB:** Amphotericin B, **CAS**:Caspofungin, **MF**:micafungin, **ANI**:Anidulafungin.
Note: The dark-colored sections in the table represent strains associated with Very Major Errors (VME), the light-colored sections indicate Major Errors (ME), while the uncolored sections reflect categorical agreement.

[14].

In this study, *C. auris* isolates were effectively distinguished on CHROMagar™ *Candida* Plus based on their distinctive colony characteristics. In addition to its use as a differential medium, this culture medium also can serve as a practical tool for the rapid presumptive identification of *C. auris* in routine laboratory settings.

Currently, *C. auris* isolates are classified into six distinct clades based on their original geographical regions: South Asia (Clade I), East Asia (Clade II), South Africa (Clade III), South America (Clade IV), Iran (Clade V), and Bangladesh (Clade VI) [15]. In our laboratory, we periodically perform clade analysis on strains identified as *C. auris* by evaluating their MALDI-TOF MS spectra using hierarchical clustering [5]. Among the group that includes the isolates from this study, no strains other than those belonging to Clade I were detected. This clade assignment may explain the relatively high antifungal resistance rates observed in our study. Consistent with earlier reports, the VITEK®2 system showed limitations in identifying Clade I isolates, In our study, 26 % of the isolates were reported as unidentified.

Fluconazole resistance was detected in 79.36 % of isolates using SYO and in 69.84 % using VITEK®2, with a categorical agreement of 55.5 %, which is lower than previously reported values [12]. This discrepancy may be associated with clade-related resistance patterns, as Clade I has been consistently reported as the most fluconazole-resistant group [15].

Inter-method discrepancies in antifungal susceptibility testing for *C. auris* should be carefully considered during clinical decision-making. The CDC's tentative breakpoint values do not define a specific cutoff for voriconazole; instead, fluconazole susceptibility is suggested as a surrogate indicator for second-generation triazoles. In our study, both Sensititre™ YeastOne™ and VITEK®2 results demonstrated elevated MICs for fluconazole, whereas no increased MIC values for voriconazole were observed with the VITEK®2 system. This discrepancy suggests that VITEK®2 should not be used as a standalone method for voriconazole susceptibility assessment in clinical decision-making. Its use in routine patient care should therefore be cautious and limited, and results should

be confirmed using reference or alternative validated methods.

Interpretation of amphotericin B (AMB) susceptibility in *Candidozyma auris* remains challenging due to the absence of species-specific clinical breakpoints established by EUCAST or CLSI. Current assessments therefore rely on the CDC's tentative breakpoint (MIC ≥2 mg/L) and epidemiological cutoff values (ECOFFs), rather than validated outcome-based criteria. In this context, AMB susceptibility results should be interpreted cautiously and considered as microbiological indicators rather than definitive predictors of clinical resistance.

According to various studies conducted in recent years, reported AMB resistance rates in *C. auris* vary widely, ranging from 8 % to 67.1 % [15–18]. In a global study by Chow et al., amphotericin B resistance was observed predominantly in clades I and IV, with significantly higher resistance rates among clade I isolates [17]. In our study, all isolates belonged to clade I, which may explain the relatively high AMB resistance rates observed.

Using the Sensititre™ YeastOne™ (SYO) method, AMB resistance was detected in 63.5 % of isolates, whereas the VITEK®2 system reported a lower resistance rate of 39.68 %. Notably, more than half of the isolates classified as resistant by SYO (34/63; 54 %) exhibited an MIC value of exactly 2 mg/L, corresponding to the CDC's tentative resistance breakpoint for *C. auris*. As the CDC criteria do not define an intermediate category, isolates at this threshold are technically categorized as resistant; however, their clustering at the cutoff value highlights the need for cautious interpretation, as these isolates may represent non-wild-type populations rather than true clinical resistance.

Recent EUCAST analyses suggest that an ECOFF of approximately 2 mg/L is appropriate for amphotericin B against *C. auris*, supporting a conservative interpretation of MIC values at this level. Method-dependent variability further complicates susceptibility assessment. In our study, the categorical agreement between SYO and VITEK®2 for AMB was low (50.8 %), and the very major error (VME) rate for VITEK®2 was high (36.5 %). For amphotericin B, the MIC_{90} value obtained with VITEK®2 was higher than that obtained with SYO. These findings are consistent with previous reports indicating that automated systems, including VITEK®2, may yield higher MIC values for amphotericin B compared with reference CLSI broth microdilution methods. Kathuria et al. similarly reported higher MIC_{50} values with VITEK®2 (8 μg/mL) than with CLSI-BMD (1 μg/mL) [19]. Taken together, these findings indicate that AMB MIC values around 2 mg/L should not be interpreted as definitive resistance in isolation. Instead, amphotericin B susceptibility results for *C. auris* should be evaluated using an ECOFF-based, method-aware approach, integrated with clinical, epidemiological, and therapeutic considerations.

Given that echinocandins are currently recommended as first-line therapy for *C. auris* candidemia in adults and children older than two months, while amphotericin B deoxycholate is reserved primarily for younger infants, accurate interpretation of AMB susceptibility remains clinically relevant. Although global echinocandin resistance rates remain below 8 % [18,20], the increasing reports of echinocandin-resistant and pan-resistant *C. auris* strains underscore the importance of reliable antifungal susceptibility testing [21,22].

In our study, no echinocandin resistance was detected. Nine isolates showed increased MICs for caspofungin due to the Eagle effect (paradoxical growth), a phenomenon characterized by regrowth at high antifungal concentrations. Since these isolates remained susceptible to micafungin and anidulafungin, they were considered pseudoresistant. The VITEK®2 system reported caspofungin and micafungin resistance in only one isolate, with categorical agreement rates of 84 % and 98,4 %, respectively. The 14,3 % very major error rate for caspofungin (9/63) was attributed to the Eagle effect, not to actual resistance.

The Eagle effect, particularly associated with caspofungin, complicates MIC interpretation in broth-based assays and may result in false resistance reporting. Although this effect is well documented in vitro, its clinical relevance remains uncertain [23,24]. Kordalewska et al. emphasized that only *C. auris* isolates harboring *FKS1* mutations are truly echinocandin-resistant [2]. Given the variability among testing methods, especially for caspofungin, routine susceptibility testing with caution is advised, or it may be omitted if not clinically essential.

For *C. auris*, the CDC's tentative antifungal breakpoints define a single cutoff value rather than a range, which may complicate result interpretation. In contrast, broth microdilution-based assays provide a wider MIC distribution and are therefore considered more reliable for nuanced susceptibility interpretation.

The Eagle effect associated with caspofungin can be detected when wide dilution ranges are tested, as implemented in the SYO method. In this context, when caspofungin resistance is observed in assays using broad dilution ranges, concurrent elevation of MIC values for other echinocandins is essential to differentiate true resistance from paradoxical growth attributable to the Eagle effect.

5. Conclusion

CHROMagar™ *Candida* Plus may be used as a practical screening tool for *Candidozyma auris* in laboratories without access to MALDI-TOF MS; however, confirmatory identification remains essential. Significant discrepancies were observed between the VITEK®2 system and the Sensititre™ YeastOne™ assay in antifungal susceptibility testing, largely attributable to the limited dilution ranges of VITEK®2, resulting in low categorical agreement and high error rates for fluconazole, voriconazole, and amphotericin B. In our study, although elevated MIC values for fluconazole were detected by both methods, no increase in voriconazole MICs was observed with VITEK®2, suggesting that VITEK®2 results for voriconazole should not be used as the sole basis for clinical decision-making. Interpretation of amphotericin B susceptibility is challenging due to the absence of species-specific clinical breakpoints. Discrepancies observed between the SYO and VITEK®2 methods indicate that MIC values around the CDC's tentative breakpoint of 2 mg/L should be interpreted cautiously, taking both methodological variability and clinical context into account. Elevated caspofungin MICs detected by SYO were associated with the Eagle effect, and preserved susceptibility to other echinocandins was essential for distinguishing true resistance. Given the lack of established clinical breakpoints, antifungal susceptibility results for *C. auris* should be interpreted cautiously and in conjunction with clinical, epidemiological, and therapeutic data.

CRediT authorship contribution statement

Gülşah Ece Özmerdiven: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Arzu İrvem:** Writing – review & editing, Formal analysis, Data curation.

Ethical approval

Our study was prepared in accordance with the Helsinki declaration.**:** The study was approved by the local Ethics Committee of Kanuni Sultan Suleyman Training and Research Hospital (ethics committee no: KAEK/2025.02.45).

Funding

There is no funding.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Reports a relationship with that includes:. Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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