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Narrative review

How to interpret MICs of amphotericin B, echinocandins and flucytosine against *Candida auris* (*Candidozyma auris*) according to the newly established European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints

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ABSTRACT

Background: *Candida auris* (*Candidozyma auris*) has emerged as an important pathogen across all continents, with clonal outbreaks and hospital transmissions. Most isolates are fluconazole resistant, and variable resistance rates are reported for amphotericin B and echinocandins.
Objectives: This study aimed to present an overview of the newly established epidemiological cut-off values (ECOFFs) and antifungal breakpoints against *C auris* and the supporting evidence.
Sources: This document is based on the recently updated European Committee for Antimicrobial Susceptibility Testing (EUCAST) rationale documents, clinical breakpoint, and ECOFF documents.
Content: An alternative approach was adopted for ECOFF setting of *C. auris* to avoid MIC distributions dominated by isogenic outbreak strains. A carefully selected strain collection of 30 isolates from 11 countries, representing five clades and 21 unique genotypes, was shared among five individual laboratories. MICs were determined with the EUCAST E.Def 7.4 methodology, providing five non-clonal datasets well above the required ≥100 total MICs per drug. Available PK-PD and clinical data were reviewed.
Implications: The following ECOFFs and breakpoints were established for *C auris*: amphotericin B: ECOFF: 2 mg/L, S: ≤0.001 mg/L, R: >2 mg/L; implying that the entire wild-type distribution is sus-

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Emerging resistance
EUCAST
Flucytosine

ceptible, increased exposure (I) (increased dose: 5 mg/kg liposomal amphotericin B daily); anidulafungin and micafungin: ECOFFs: 0.25 mg/L, S: ≤0.25; R: >0.25; rezafungin: ECOFF: 0.125 mg/L; and flucytosine: ECOFF: 0.5 mg/L. Importantly, notable MIC variations have been reported for *C auris* and some agents across commercial tests. Consequently, important detailed guidance is provided on how to validate your MIC test in-house before adopting the EUCAST breakpoints for MIC interpretation. **Maiken Cavling Arendrup, Clin Microbiol Infect 2026;32:56**

Introduction

Candida auris (recently renamed as *Candidozyma auris*) has emerged as an important pathogen during the last decade. It was first described after being isolated from the ear canal of a Japanese patient in 2009 but remained under cover until notable outbreaks were reported from India [1] and later from all continents [2]. It is phylogenetically related to the *Candidia. haemulonii* (*Candida haemuli*) clade and intrinsically echinocandin susceptible. However, the acquisition of echinocandin resistance due to mutations in the target genes (hot spots 1 and 2, and less commonly in hot spot 3 of the *fks1* gene) can occur rapidly (e.g. within 2 weeks of therapy) [3–5]. Although the fluconazole susceptibility of the earliest isolated *C auris* strains (and likely representing the wild-type, CBS10913) was low (4 mg/L by European Committee for Antimicrobial Susceptibility Testing [EUCAST] tested in-house), the majority of *C auris* isolates today have high MICs to fluconazole, many with resistance mutations in the *ERG11* gene [6], and variable MICs to the other azoles [7–9]. However, *C auris* isolates with low azole MICs are found particularly in South America [10,11].

The CDC issued treatment recommendations for *C. auris* infections [12]. First-line therapy is an echinocandin for both adults and children >2 months. Retrospective studies showed that anidulafungin, caspofungin, and micafungin are equally efficacious [13]. Second-line therapy is liposomal amphotericin B at an elevated dose (5 mg/kg daily, vs. the standard dose of 3 mg/kg daily) and is recommended when susceptibility testing indicates echinocandin resistance or when patients treated with echinocandins do not improve after 5 days [12]. Finally, for infants aged <2 months, amphotericin B deoxycholate at 1 mg/kg daily is the initial recommended treatment, with liposomal amphotericin B as the second-line option again at an elevated (5 mg/kg daily) [12].

Clinical outcome data on the use of elevated doses of liposomal amphotericin B are scarce. This is partly because echinocandins are the first-line treatment, resulting in limited use. However, another contributing factor is the notable variation documented in amphotericin B susceptibility testing, particularly with several commercial tests [14–19]. The challenges associated with susceptibility testing, combined with the fact that MICs of amphotericin B against *C auris* are higher than those against *Candidia glabrata (Nakaseomyces glabratus)* and *Candidia krusei (Pichia kudriavzevii)*, create issues when a nonspecies-specific cut-off of ≤1 mg/L is used for susceptibility interpretation. This threshold bisects the wild-type distribution, leading to inconsistent susceptibility classification of wild-type isolates and artificially elevated resistance rates [14–19]. These challenges have contributed to reluctance in using amphotericin B and highlight the need for robust MIC testing and clinical breakpoints that do not bisect the wild-type distribution.

In 2025, the EUCAST Antifungal Susceptibility Testing Subcommittee (EUCAST AFST) has set epidemiological cut-off values (ECOFFs) for amphotericin B, echinocandins, and flucytosine against *C auris*. With the CDC treatment recommendations and very few treatment options, EUCAST AFST has also proposed clinical breakpoints for some of these agents to facilitate optimal therapy. These will apply to susceptibility testing performed according to the EUCAST AFST reference method and to other tests if thoroughly validated and confirmed to generate similar MIC values (see specific recommendations for method validation below).

Challenges faced when setting ECOFFs and clinical breakpoints for *C auris*

Several factors are considered by EUCAST when clinical breakpoints are established. These include dosing information, MIC distributions, ECOFFs, preclinical and clinical pharmaco/pharmacodynamics (PK/PD), Monte Carlo simulations, PK/PD cut-off values and clinical data [20]. For ECOFF setting, at least five datasets, each consisting of at least 15 MICs against individual isolates, in total comprising at least 100 MICs, must be included, with the modal MIC within ±1 two-fold dilution from the most common modal MIC. Moreover, the acceptance criteria state that each distribution has to be unique and not outbreak-related. This latter important point effectively disqualifies almost all laboratory MIC data, because the epidemiology of *C. auris* infection is dominated by clonal outbreaks that would lead to bias when setting an ECOFF. Hence, EUCAST AFST adopted a novel approach when generating MIC data for ECOFF setting. This approach involved using a carefully selected *C auris* strain collection of 30 isolates, representing five clades, derived from 11 countries and confirmed to be highly genetically diverse with 21 different short tandem repeat genotypes (Table S1) [21]. This collection was subsequently shared and MIC tested with the EUCAST E.Def 7.4 methodology in five different laboratories, thereby providing five non-clonal datasets and complying with the requirement of >100 MICs per agent in total (see Table 1).

Amphotericin B

Breakpoints established. EUCAST has set breakpoints for amphotericin B against *C auris* classifying the entire wild-type population as susceptible, increased exposure (I), which applies to a liposomal amphotericin B dose of 5 mg/kg daily (Table 2).

Background. Amphotericin B is licensed for treatment of systemic or severe *Candida* and *Aspergillus* infections (and other fungal infections). Whereas the recent global guidelines for the treatment of candidiasis do not include *C auris* specifically [22], the CDC recommends liposomal amphotericin B as second-line option for *C auris*, provided an elevated dose is used (5 mg/kg daily, rather than a standard dose of 3 mg/kg daily) [12]. Given the widespread azole resistance of *C auris* and the limited number of antifungal drug classes available, no other currently licensed agents are available as alternatives to echinocandins for the treatment of *C auris*. The two most commonly used formulations of amphotericin B are conventional amphotericin B deoxycholate (C-AMB)

Table 1
Combined MIC distributions for amphotericin B, echinocandins, and flucytosine (each consisting of distributions from five centres) against *C. auris*

Antifungal agent	*N*	MIC (mg/L)																		ECOFF (mg/L)
		0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	
Amphotericin B	150							6	22	*55*	**64**	3									2
Anidulafungin	150			8	21	***54***	43	12	6	3	2	1									0.25
Micafungin	150			1	8	43	***70***	21	4	3											0.25
Rezafungin	149	2	6	22	44	***50***	17	5	2		1										0.125
Flucytosine	150						11	***66***	55	18											0.5

The ECOFFs were determined as consensus ECOFFs based on the eyeball method (visual inspection of the MIC histograms) and the ECOF Finder programme including 95%, 97.5%, 99%, 99.5%, and 99.9% of the MICs, and ratified after public consultation. The mode is highlighted in bold, and the MIC_{50} in italic font.
ECOFF, epidemiological cut-off values.

standard dose 0.75-1 mg/kg daily and liposomal amphotericin B (L-AMB) standard dose 3 mg/kg daily.

Considerations related to breakpoints. The EUCAST ECOFF for amphotericin B against *C auris* is 2 mg/L, one dilution higher than ECOFFs against most other *Candida* spp. (Table 1). The broth microdilution MIC distributions are narrow and comparable across EUCAST and Clinical and Laboratory Standards Institute [16–18]. The MICs obtained using gradient tests are wider and sometimes bimodal [16]. This has been ascribed to a significant correlation between the growth rate of the isolate in broth microdilution control wells and the MIC obtained in separate microdilution or gradient MIC testing [16]. An increased growth rate is expected to affect the MIC more for a diffusion test than in a microdilution test where the cut-off is relative to the internal growth control. The PK/PD relationship for amphotericin B against *C auris* is limited. Forgacs et al. [23] investigated C-AMB at 1 mg/kg i.p. daily in a neutropenic murine disseminated infection model and found a success rate of 4/10 mice. Herrada et al. [24] and Hager et al. [25] used the same mouse model and a *C auris* isolate with a high Clinical and Laboratory Standards Institute MIC of 4 mg/L and found that L-AMB dosed at 7.5 mg/kg (which gives an exposure equivalent to 5 mg/kg in humans) resulted in 90% survival and significant kidney burden reduction vs. control and vs. the use of L-AMB at doses of 3.5 and 5 mg/kg and C-AMB at doses of 0.35 mg/kg and 0.75 mg/kg daily. Lepak et al. [26] found that the PK/PD breakpoint of C-AMB was 1-1.5 mg/L for stasis using a neutropenic mouse model, and Beredaki et al. [27] found that a 95% probability of target attainment was 2 mg/L (and thus included the wild-type population) for L-AMB dosed at 5 mg/kg daily, thus both suggesting that increased dosage is needed. Clinical data are sparse. Sokou et al. [28] reported 2 of 2 successes with amphotericin B treatment in neonates vs. 6 of 9 and 8 of 14 with echinocandins and azoles, respectively. For patients receiving combination therapy including amphotericin B, 11 of 11 had successful outcomes compared to 6 of 10 patients receiving azoles combined with echinocandins. Obviously, the animal and *in vitro* PK/PD and clinical data are limited. However, with the recommendation of an elevated dose, the available data support the classification of the wild-type population of *C auris* as amphotericin B susceptible, increased exposure (I) (S $\leq$ 0.001 mg/L, R > 2 mg/L).

Table 2
European committee on antimicrobial susceptibility testing breakpoints for *C auris*

	S: $\leq$	R: >
Amphotericin B	0.001[a]	2
Anidulafungin	0.25	0.25
Micafungin	0.25	0.25
Rezafungin	IE[b]	IE
Flucytosine	IE[b]	IE

I, susceptible, increased exposure; IE insufficient evidence; R, resistant; S, susceptible.
[a] The entire *C. auris* wild-type population is in the I category. MICs against *C auris* should be interpreted as resistant when >2 mg/L. Susceptible category (0 $\leq$ .001 mg/L) is to avoid misclassification of "I" as "S".
[b] IE, insufficient evidence. European committee on antimicrobial susceptibility testing has refrained from setting clinical breakpoints for rezafungin and flucytosine against *C. auris*, as there is no available clinical experience for patients with *C. auris* treated with these agents.

Echinocandins

Breakpoints established. EUCAST has set breakpoints for anidulafungin and micafungin against *C auris* (for both agents: S: $\leq$ 0.25; R: > 0.25 mg/L; Table 2). Isolates classified as anidulafungin and micafungin susceptible (S), can be reported as caspofungin S. Isolates with discrepant classification to anidulafungin and micafungin (e.g. anidulafungin S and micafungin R), should be further analysed with target gene sequencing, as such isolates may harbour weak mutations causing a discrete loss of susceptibility [29]. EUCAST has set ECOFFs, but not breakpoints, for rezafungin, because there is no clinical experience yet for the use of this new agent for *C auris* infections. This is true both with respect to efficacy but also with respect to risk of selection of echinocandin resistance in *C. auris* given the long half-life of rezafungin, which may be associated with a longer-term selection pressure on colonising *C auris* as has been observed for other *Candida* species following echinocandin therapy [30].

Background. Anidulafungin, caspofungin and micafungin are first-line agents for *C auris* infections at standard dosing. Echinocandin resistance is caused by target gene mutations in hot spot regions of *fks1* for all *Candida* species, and for *C glabrata* additionally in *fks2* [31]. Mutations can arise as early as within 2 weeks and the impact on the MIC depends on the codon involved and specific alteration (substitution/deletion) [3,4]. There is insufficient data to define a safe level of MIC elevation that will not affect clinical outcomes.

Considerations related to breakpoints. The *in vitro* activity of anidulafungin and micafungin against *C auris* is very similar, with MIC_{50} values of 0.06 mg/L for both, modal MICs of 0.03 and 0.06 mg/L, and ECOFFs of 0.25 mg/L for both (Table 1). MICs of rezafungin are slightly lower (ECOFF 0.125 mg/L), which is likely attributable to the fact that rezafungin MIC testing involves supplementation of the growth medium with Tween 20 to prevent drug loss due to rezafungin binding to plastics during plate preparation and within the plate itself [32,33]. Pharmacodynamic targets for anidulafungin and micafungin against *C auris* or *Candida* spp. in general have not been determined using the EUCAST method. For rezafungin, using stasis as the endpoint, the probability of achieving the non-clinical PK/PD targets associated with net fungal stasis for *C auris* was determined. Based on the PK/PD target 127 $fAUC_{0\text{-}168}$/MIC, a $\geq$95% target attainment rate was found for *C auris* isolates with MICs $\leq$0.06 mg/L for the standard dosing of rezafungin (400 mg on Day 1, followed by 200 mg on Day 8 and 200 mg weekly thereafter, with no elevated

dose licensed). In a recent retrospective clinical study involving 72 patients, clinical cure rates (71%/73%/67%), microbiological cure rates (84%/88%/80%), and mortality at Day 28 (29%/27%/58%) were reported for micafungin, caspofungin, and anidulafungin, respectively, with no significant difference among the treatment arms [13]. Noticeably, a significantly higher proportion of patients had chronic liver disease in the anidulafungin group. Based on these data and the fact that echinocandins are recommended as first-line agents, with notable clinical experience beyond published studies suggesting their efficacy, EUCAST has set clinical breakpoints that include the wild-type population as susceptible to anidulafungin, micafungin, and caspofungin (the latter based on anidulafungin and micafungin susceptibility).

Flucytosine

ECOFFs established. EUCAST has set ECOFFs for flucytosine and *C auris* (ECOFF 0.5 mg/L, Table 1).

Background. Flucytosine is an antifungal agent originally developed for cancer chemotherapy but today used primarily in combination with amphotericin B against cryptococcal meningoencephalitis, due to the synergistic activity between the two. Less often flucytosine is used for other *Candida* infections affecting difficult to reach foci (such as endocarditis, osteomyelitis, or endophthalmitis) [22]. Flucytosine is a small molecule, highly water soluble, and with very good penetration into tissues including the brain. It is never used as monotherapy because resistance develops quickly. It is also not part of standard care for patients with *C. auris*. However, given its good *in vitro* activity against *C. auris*, the absence of antagonism when used in combination with other antifungals [34], and the limited treatment options in general for this organism, it may be useful to use as part of combination therapy in selected cases provided the isolate is wild-type.

Considerations related to breakpoints. In the global guideline for the diagnosis and management of candidiasis just released, the use of flucytosine in combination with amphotericin B or another antifungal received an AIII (strong) recommendation in patients with central nervous system (CNS) infection, and a CIII (weak) recommendation in patients with *Candida* endocarditis, and in patients with fluconazole resistant *Candida* urinary tract infections [22]. Of note, this global guideline has limitations, e.g. by not covering emerging pathogens such as *C auris*, and thus the use of flucytosine in the setting of *C auris* was less thoroughly discussed. On this background, we have refrained from setting clinical breakpoints but provided an ECOFF to allow understanding of whether the isolate is wild-type or may have acquired resistance.

Conclusion

The EUCAST AFST acknowledges the need for MIC interpretation for the emerging pathogen *C. auris* and hereby provides ECOFFs and/or clinical breakpoints for amphotericin B, anidulafungin, micafungin, and flucytosine based on the current knowledge. An alternative approach has been adopted for ECOFF setting to avoid biased MIC data resulting from the potential inclusion of isogenic strains if unselected MICs data set from outbreaks were used. Given the notable variation in MICs obtained, particularly for amphotericin B and *C auris* when using many commercial AFST tests (see warning issued at the EUCAST.org website), it is crucial that the ECOFFs and breakpoints are only adopted after verifying that in-house testing of these agents against *C auris* aligns with the EUCAST methodology. We recommend that this is performed in a two-step process. First, each EUCAST *Candida* QC strain should be tested 10 times for each of the agents. The mode should align with the target and the MICs should fall within the range. Random variation is permissible (maximum 1/10 MIC value outside the range), but systematic deviation (i.e. the mode consistently falling to one side of the target) is not acceptable. Second, if the above criteria are met, we recommend to test 10 clinical isolates of each of the following species: *C albicans, C auris, C glabrata, C krusei,* and *C tropicalis*. The modal MICs for all six species should be within ±1 dilution of the mode for each drug-bug combination found in the EUCAST rationale documents, available on the EUCAST website (https://www.eucast.org/astoffungi/rationale_documents_for_antifungals/). If this criterion is also met, there is a high likelihood of achieving an accurate MIC and correct interpretation when applying the EUCAST breakpoints.

Before publication, these ECOFFs and breakpoints have undergone approval by the EUCAST Steering Committee and a public consultation. We hope that these recommendations are well received and help ensure appropriate therapy for patients with *C auris* infections.

Author's contributions

Conceptualization M.C.A., J.G., S.A-A., C.G.G., and J.M. Methodology and validation: M.C.A., J.G., S.A-A., E.F.J.M., and J.M. Resources: M.C.A., J.G., S.A-A., E.F.J.M., J.F.M., and J.M. Formal analysis, investigation, data curation: M.C.A., J.G., S.A-A., E.F.J.M., and E.D. (supporting), J.F.M. (supporting), J.B.B. (supporting), and J.M. (lead). Writing original draft: M.C.A. Writing—review and editing: all authors and Subcommittee members. Funding acquisition: M.C.A. and J.M.

Transparency declaration

Potential conflict of interest

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Outside this study the main authors declare the following for the past 36 months: M.C.A. received grants or contracts paid to the institution from Scynexis, Pfizer, F2G, Shionogi, Cidara, and Mundipharma. She has received an honorarium for one talk from each of the following companies: F2G/Shionogi, and Gilead, and she is the current chair of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) antifungal subcommittee. J.G. has received funds for participating in educational activities organised on behalf of Gilead, Pfizer, and Mundipharma; he has also received research funds from FIS, Gilead, F2G, Scynexis, Mundipharma, and Cidara. S.A-A. received research grant from Cidara and lecture honoraria from Gilead. E.F.J.M. received research grants form Mundipharma and Scynexis, is in the scientific advisory board for Pfizer and has received speaker fees from Gilead Sciences. J.B.B. has received research funds from Biomérieux, honoraria for participation in an advisory committee from, Shionogi and Gilead and for delivering a lecture from Pfizer, all paid to the institution. E.D. has received grants from Biomérieux, honoraria for lectures from Pfizer and Mundipharma, and supports for attending meetings from Gilead, Pfizer, and Mundipharma. J.M. has research contracts paid to the institution by Gilead, Pfizer, Cidara, and Mundipharma. G.G.G., P.L., T.M., and J.F.M. have nothing to declare. O.K. received grants or reagent supply to the institution from Bosch, Mast Diagnostica, Virotech, Basilea, and Pfizer; honoraria for talks or scientific counselling paid to institution from BG-RCI (professional association for raw materials and chemical industry), Fujifilm Wako, Gilead, Pfizer, and Mundipharma. C.L.F. received grants or contracts paid to the institution from Scynexis, Pfizer, F2G,

Shionogi, Gilead, and Mundipharma. She has received an honorarium for one talk from each of the following companies: Pfizer, Shionogi, Gilead, and Mundipharma. J.W. has received a research grant from Pfizer, lecture honoraria from Gilead and Fujifilm Wako, a honorarium for participation in an advisory committee from Mundipharma, support for attending a meeting from Mundipharma, and financial support paid to the institution for organising educational symposia from Pfizer, Mundipharma, and A. Menarini. K.A., N.F., L.R.F., G.V., J.V.B. have nothing to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.07.002.

Appendix 1. EUCAST-AFST:

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