

Rationale for EUCAST clinical breakpoints

Agent	Amphotericin B	
Current version	3.0	26 th June 2025
Previous versions	2.0	4 th February 2020 (Amphotericin B)
	1.0	19 th November 2010 (Amphotericin B vs <i>Candida</i> spp.)
	1.0	15 th July 2011 (Amphotericin B vs <i>Aspergillus</i> spp.)

Foreword

EUCAST

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is organised by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Centre for Disease Prevention and Control (ECDC), and the active national antimicrobial breakpoint committees in Europe. EUCAST was established by ESCMID in 1997, was restructured in 2001-2002 and has been in operation in its current form since 2002. The current remit of EUCAST is to harmonise clinical breakpoints for existing drugs in Europe, to determine clinical breakpoints for new drugs, to set epidemiological (microbiological) breakpoints, to revise breakpoints as required, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance. Information on EUCAST and EUCAST breakpoints is available on the EUCAST website at <http://www.EUCAST.org>.

EUCAST rationale documents

EUCAST rationale documents summarise the information on which the EUCAST clinical breakpoints are based.

Availability of EUCAST document

All EUCAST documents are freely available from the EUCAST website at <http://www.EUCAST.org>.

Citation of EUCAST documents

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1. Introduction

Amphotericin B is a polyene antifungal agent with *in vitro* activity against most yeasts and moulds. It is available in three different formulations including amphotericin B deoxycholate (D-AmB) and two lipid formulations: amphotericin B lipid complex (ABLC) and liposomal amphotericin B (L-AmB). The active compound is identical but the pharmacokinetics and toxicity profiles are different from formulation to formulation. The three formulations of amphotericin B are licensed for treatment of systemic or severe *Candida* and *Aspergillus* infections (and other fungal infections), and empirical therapy for presumed fungal infection in febrile, neutropenic patients, although licensed applications may differ from country to country where local licensed indications should be consulted.

The mould species most frequently causing human invasive aspergillosis include *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus niger*. Although *Aspergillus* species are generally susceptible to polyenes, elevated MICs have been reported for some species including *A. flavus*, *A. terreus*, *A. nidulans*, *A. lentulus* and *A. fumigatiaffinis*. Amphotericin B has limited activity against *A. terreus*, *A. flavus*, and against *A. nidulans* in the setting of chronic granulomatous disease.

The *Candida* species most frequently involved in causing human infections include *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*. The *in vitro* activity of amphotericin B against species of *Candida* is mostly uniform including against *Candida auris* (1). Moreover, it seems that acquired resistance to amphotericin B in *C. auris* is associated with a fitness-cost (2). Amphotericin B has limited clinical activity against *Candida lusitanae* although the MICs are comparable to those for the other *Candida* spp. This is due to a higher mutational rate and less fungicidal activity when exposed to amphotericin B.

The European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST) determined breakpoints for amphotericin B against *Aspergillus* spp. and *Candida* spp. in 2011 and 2010, respectively.

Version 2.0 of this rationale document, combined the *Aspergillus* and *Candida* documents, included ECOFFs for *Cryptococcus neoformans* and enriched MIC distributions for *Candida* spp., *Aspergillus* spp., and *Cryptococcus neoformans*. Finally, the R breakpoint was lowered for all species due to the revised definition of the I category (Susceptible, Increased exposure).

In version 3.0 of this rationale document, MIC distribution, ECOFFs and breakpoints have been included for *Candida auris*.

2a. Dosage for treatment of aspergillosis / candidiasis*: D-AmB

	Denmark	Spain	Sweden	Switzerland	Turkey	Austria	Norway	France	The NL	The UK
Minimum dose (mg/kg/day)	1/0.7-1		1/0.3	NA/NA	1/0.5	1/0.5	0.5	1/NA	1/0.5	0.25
Most common dose (mg/kg/d)	1-1.5/0.7-1		1-1.5/0.7	1.5/0.5-1	1-1.5/1-1.2	1-1.5/1-1.5	1-1.5/0.75	1-1.5/0.5-1	1-1.5/1	0.25-1
1 st day dose (mg/kg/day)	0.5/0.25		0.5/0.25	0.5/0.25	0.5/0.25	0.5/0.25	0.5/0.25	0.5/0.25	0.5/0.25	0.25
2 nd day dose (mg/kg/day)	1/0.5		1/0.5	NA/NA	NA/NA	NA/NA	1/0.5	NA/NA	1/0.5	NA
Maximum dose (mg/kg/d)	1.5/1-1.5		1.5/1	1.5/1.5	1.5/1.5	1.5/1.5	1.5/1	1.5/1	1.5/1.5	1.5/1.5
Loading dose (mg/kg/d)	1/1		NA/NA	0.25/0.25	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	0.25
Available formulations	IV	None	IV, oral 10 mg tablet**	IV	IV	IV	IV	IV, oral 250 mg tablets 10% oral suspension**	IV	IV

* For *Cryptococcus meningitis* (and mucormycosis) appropriate dosages please visit management guidelines for these infections

**oral formulations are not absorbed but used for oral gut decontamination only. NA = Not applicable. IV = Intravenous.

2b. Dosage for treatment of aspergillosis / candidiasis: L-Amb^B*

	Denmark	Spain	Sweden	Switzerland	Turkey	Austria	Norway	France	The NL	Italy	The UK
Minimum dose (mg/kg/day)	3	1	1	1	1	1	1	NA	3	3	1
Most common dose (mg/kg/d)	3	3	3	3	3-5	3-5	3	3	3	3-5	3
1 st day dose (mg/kg/day)	3	1	3	1	1	1	1	1	1	3	1
2 nd day dose (mg/kg/day)	3	3	3	3	1	1	1	1	3	3	1-3
Maximum dose (mg/kg/d)	5, 7, and 10	5	3	5-6	5	5	5	10	5	5-10	NA
Loading dose (mg/kg/d)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Available formulations	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV	IV

*Dosages for treatment of patients with aspergillosis and candidiasis are identical. Maximum dosages are used for serious/non-responding/CNS cases of aspergillosis, candidiasis and cryptococcosis, as well as for mucormycosis. NA = Not applicable. IV = Intravenous.

2c. Dosage for treatment of aspergillosis / candidiasis: ABLC

	Denmark	Spain	Sweden	Switzerland	Turkey	Austria	Norway	France	The NL	Italy	The UK
Minimum dose (mg/kg/day)		3	NA			3	5	NA	3	3	5
Most common dose (mg/kg/d)		5	5			3-5	5	5	3-5.5	5	5
1 st day dose (mg/kg/day)		5	5			5	NA	NA	3-5.5	5	5
2 nd day dose (mg/kg/day)		5	5			NA	NA	NA	3-5.5	5	5
Maximum dose (mg/kg/d)		5	5			5	5	5	5.5	5	5
Loading dose (mg/kg/d)		NA	NA			NA	NA	NA	NA	NA	NA
Available formulations	None	IV	IV	None	None	IV	IV	IV	IV	IV	IV

Cells are left empty when data not readily available or when the compound is not available in that country. Dosages for treatment of patients with aspergillosis and candidiasis are identical. NA = Not applicable. IV = Intravenous.

3a. MIC distributions (numbers) and epidemiological cut-off (ECOFF) values (mg/L)

	N	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	(T)ECOFF*	
<i>Aspergillus flavus</i>	207								6	42	83	59	15	1	1							4
<i>Aspergillus fumigatus</i>	2627				1	1	11	384	593	1216	223	12	1									1
<i>Aspergillus nidulans</i>	121						2	4	8	37	38	20	4	3	5							(4) ^a
<i>Aspergillus niger</i>	185				1	6	27	50	70	28	2		1									(0.5)
<i>Aspergillus terreus</i>	188								3	11	42	69	48	12	3							8
<i>Fusarium fujikuroi</i>	99							2		2	13	41	29	11	1							(8)
<i>Fusarium solani</i>	72			1						5	33	19	6	7	1							(8)
<i>Candida albicans</i>	1342			1	2	13	59	518	506	223	19	1										1
<i>Candida auris</i>	150							6	22	55	64	3										2
<i>Candida dubliniensis</i>	146				2	41	61	26	10	6												0.25
<i>Candida glabrata</i>	907					9	13	215	417	230	22	1										1
<i>Candida guilliermondii</i>	88					8	21	38	17	4												(0.5)
<i>Candida krusei</i>	262							6	35	167	53	1										1
<i>Candida parapsilosis</i>	314					5	8	26	116	141	17					1						1
<i>Candida tropicalis</i>	257						4	31	134	81	7											1
<i>Candida kefyr</i>	64						1	11	19	26	7											(1)
<i>Candida lusitanae</i>	59					2	21	17	14	5												(0.5)
<i>Saccharomyces cerevisiae</i>	81				1	2	9	21	37	10	1											(0.5)
<i>Cryptococcus neoformans</i>	1022				1	12	82	371	406	123	25	2										(1)
<i>Cryptococcus gattii</i>	52					2	11	25	13	1												(0.5)

The table includes EUCAST MIC distributions available at the time breakpoints were set. They represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. Of note, the level of species identification varies across laboratories. Consequently, ID may likely include cryptic species.

* tentative ECOFF (TECOFF) was determined because <5 qualified MIC distributions were aggregated, and is indicated in parenthesis.

^a weighted ECOFF was determined because one centre contributed >50% of all MICs.

3b. MIC distributions (%)# and epidemiological cut-off (ECOFF) values (mg/L)

	N	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	(T)ECOFF*	
<i>Aspergillus flavus</i>	207								3	20	40	29	7									4
<i>Aspergillus fumigatus</i>	2627							15	23	46	8											1
<i>Aspergillus nidulans</i>	121						2	3	7	31	31	17	3	2	4							(4) ^a
<i>Aspergillus niger</i>	185				1	3	15	27	38	15	1		1									(0.5)
<i>Aspergillus terreus</i>	188								2	6	22	37	26	6	2							8
<i>Fusarium fujikuroi</i>	99							2		2	13	41	29	11	1							(8)
<i>Fusarium solani</i>	72			1						7	46	26	8	10	1							(8)
<i>Candida albicans</i>	1342					1	4	39	38	17	1											1
<i>Candida auris</i>	150							4	15	37	43	2										2
<i>Candida dubliniensis</i>	146				1	28	42	18	7	4												0.25
<i>Candida glabrata</i>	907					1	1	24	46	25	2											1
<i>Candida guilliermondii</i>	88					9	24	43	19	5												(0.5)
<i>Candida krusei</i>	262							2	13	64	20											1
<i>Candida parapsilosis</i>	314					2	3	8	37	45	5											1
<i>Candida tropicalis</i>	257						2	12	52	32	3											1
<i>Candida kefyr</i>	64						2	17	30	41	11											(1)
<i>Candida lusitanae</i>	59					3	36	29	24	8												(0.5)
<i>Saccharomyces cerevisiae</i>	81				1	2	11	26	46	12	1											(0.5)
<i>Cryptococcus neoformans</i>	1022					1	8	36	40	12	2											(1)
<i>Cryptococcus gattii</i>	52					4	21	48	25	2												(0.5)

Percentage values are rounded to nearest whole number. Consequently, the sum can deviate slightly from 100%.

The table includes EUCAST MIC distributions available at the time breakpoints were set. They represent combined distributions from multiple sources and time periods. The distributions are used to define the epidemiological cut-offs (ECOFF) and give an indication of the MICs for organisms with acquired or mutational resistance mechanisms. They should not be used to infer resistance rates. Consequently, ID may likely include cryptic species.

* tentative ECOFF (TECOFF) was determined because <5 qualified MIC distributions were aggregated, and is indicated in parenthesis.

^a weighted ECOFF was determined because one centre contributed >50% of all MICs.

4. Pharmacokinetics								
	Amphotericin B deoxycholate			Amphotericin B lipid complex		Liposomal amphotericin B ²		
Patients group (Reference)	HIV patients (3)	Healthy (4)	Healthy (5)	HIV patients (3)	Immunocompromised (3)	Allo-HSCT (6)	Immunocompromised (7)	
Dosage (mg/kg/day)	0.3	0.6	1	1.2	5	3	7.5	10
C _{max} (mg/L)	0.72 ± 0.22	1.43 ± 0.2	2.83 ± 1.17	2.72 ± 0.96	2.39 ± 1.58	21.87 ± 12.47	115.1 ± 104.9	164.7 ± 119.7
C _{min} (mg/L)		0.25 ± 0.03				10.63 ± 11.48		
Total body clearance/F (L/h/Kg)	9.45 ± 4.18	0.91	2.31	4.69 ± 2.94	0.27 ± 0.07	1.03 ± 0.53	1.05 ± 0.77	0.84 ± 0.84
Distribution, elimination, and terminal t _{1/2} (h)	190.70 ± 141.01	0.17 ± 0.14, 6.8 ± 1.6, 127 ± 30	0.64 ± 0.24, 15.23 ± 5.25, NA	144.31 ± 136.85	393 ± 486	0.56 ± 0.48, 6.0 ± 2.1, 152 ± 116	ND, 6.0 ± 0.8, ND	ND, 8.4 ± 2.6, ND
AUC _{0-24h} (mg.h/L)	6.14 ± 0.65	13.9 ± 2.0	28.98 ± 15.46	4.38 ± 0.66	19.17 ± 4.43	96.6 ± 30.9	1,333 ± 2,153	1,919 ± 2,056
% Fraction unbound ¹ (% CV)	4.7-0.8 ¹ (25% at fC _{max})					Non-liposomal (protein bound + free AMB) = 2 ± 1.4% at the end of infusion-45 ± 10% on 7d, (<36%)		
Volume of central compartment, V _c (L/kg)	1.8 ± 0.38	0.136 ± 0.06	0.33 ± 0.15	7.93 ± 1.12	147 ± 144	0.26 ± 0.08	0.14 ± 0.10	0.16 ± 0.17
Comments	¹ The protein binding of amphotericin B is concentration-dependent with the upper solubility limit in plasma <1 mg/L (4). ² Pharmacokinetics may vary with dosages >7.5 mg/kg Cells are left empty when data are not readily available							

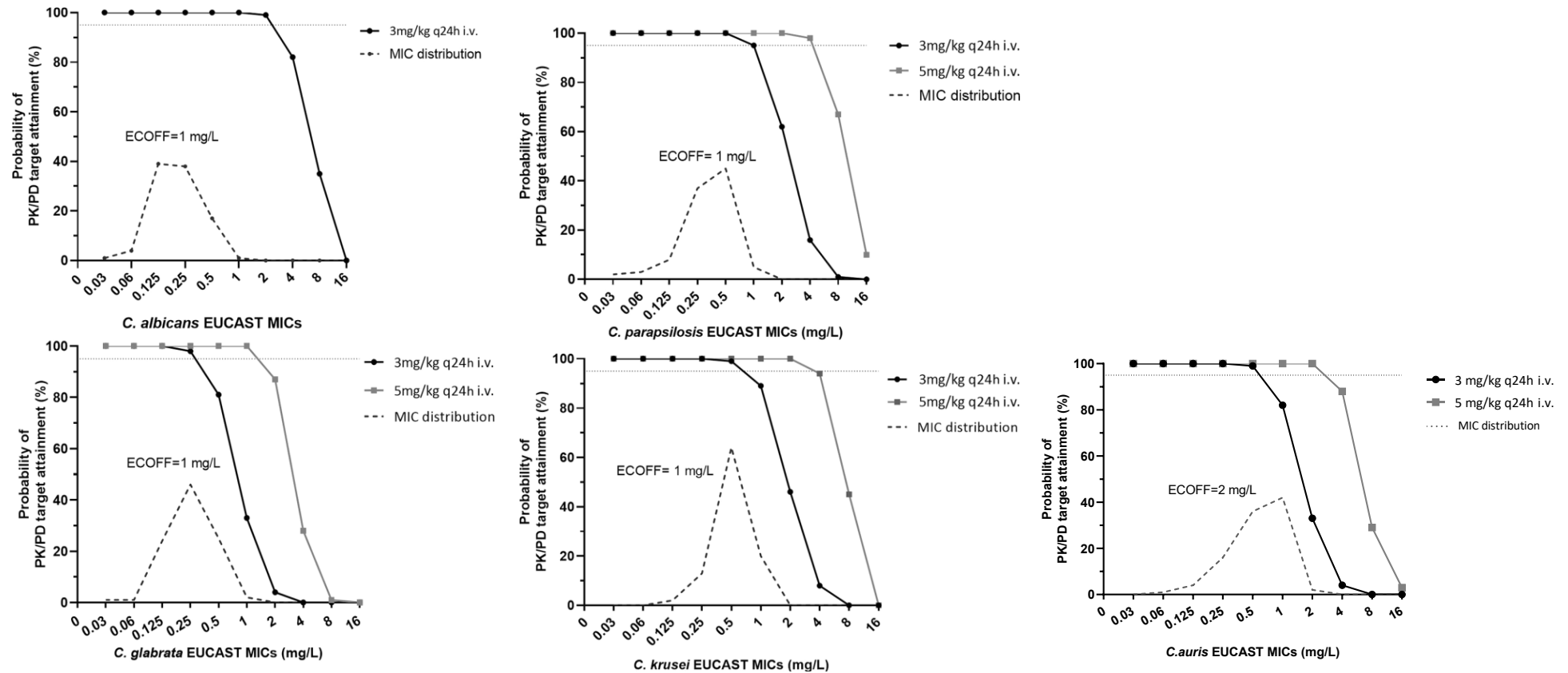
5. Pharmacodynamics			
	<i>Aspergillus</i> spp. (N of strains)	<i>Candida</i> spp. (N of strains)	<i>Cryptococcus</i> spp. (N of strains)
Fungistatic/1-log kill dose (mg/kg) for fungal burden in kidney in neutropenic murine model of disseminated candidiasis (8), range, CLSI		<i>C. albicans</i> (N=3) (MIC= 0.25 mg/L) D-AmB= 1.86-4.46/2.70->20 <i>C. krusei</i> (N=1) (MIC= 0.25 mg/L) D-AmB= 1.94/8.01 <i>C. dubliniensis</i> (N=1) (MIC= 0.5 mg/L) D-AmB= 3.60/>20	
ED₅₀/1-logkill dose (mg/kg) for fungal burden in kidney in neutropenic murine model of disseminated candidiasis (9), range, CLSI		<i>C. albicans</i> (N=5) (MIC=0.25 mg/L) D-AmB= 0.15-1.49/2.09-11.8 L-AmB= 2.63-3.94/11.40-28.5 ABLC= 0.58-4.11/3.45-45	
C_{max}/MIC for stasis for fungal burden in an <i>in vitro</i> PK-PD model (8, 10, 11), mean (95% CI), EUCAST		<i>C. albicans</i> (N=1) (MIC=0.25 mg/L) L-AmB= 2.1 (0.5–3.9) <i>C. glabrata</i> (N=4) (MIC=0.125-1 mg/L) L-AmB= 24 (18–32) <i>C. parapsilosis</i> (N=2) (MIC=0.25-0.5 mg/L) L-AmB= 8 (5–15) <i>C. krusei</i> (N=2) (MIC=0.5-1 mg/L) L-AmB= 10 (5-18) <i>C. auris</i> (N=4) (MIC=0.25-1 mg/L) L-AmB= 12 (9-16)	
ED₅₀/ED₈₀ dose (mg/kg) for 14d survival in a murine non-neutropenic model of disseminated aspergillosis (12), range, CLSI	<i>A. fumigatus</i> (N=4) (MIC=0.5 mg/L) L-AmB= 0.08-0.60/1.26-17.4		
ED₅₀ dose (mg/kg) for 14d survival in a murine neutropenic/immunosuppressed model of disseminated aspergillosis (13), range, CLSI	<i>A. fumigatus</i> (N=2) (MIC=0.5 mg/l) L-AmB= 1.4-1.92/2.40-2.56		
ED₅₀ dose (mg/kg) for 15d survival in a murine non-neutropenic murine model of disseminated aspergillosis (14), mean (95% CI), CLSI	<i>A. fumigatus</i> (N=1) (MIC=1 mg/L) D-AmC= >1 L-AmB= 0.06 (0.03-0.127) ABLC= 0.21 (0.06-0.66)		
AMB total plasma concentrations (mg/L) produce 50% of maximal galactomannan suppression/killing rate in neutropenic rabbit model of pulmonary aspergillosis (15), CLSI	<i>A. fumigatus</i> (N=1) (MIC=1 mg/l) D-AmB= 5.937/0.854 ABLC= 3.437/1.088 L-AmB= 49.272/4.874		

AUC/MIC associated with near maximal activity of fungal load in brain of a murine model of cryptococcal meningoencephalitis (16), CLSI			<i>C. neoformans</i> var. <i>grubii</i> (N=1) L-AmB= ~100
Comments	<ul style="list-style-type: none"> Because pharmacodynamic data using the EUCAST method is sparse, data from the CLSI broth microdilution method have been included. Importantly, MICs from one method cannot be directly extrapolated to another. However, EUCAST and CLSI MICs for <i>C. auris</i> tend to be within one two-fold dilution, whereas other methods tend to provide higher and wider MIC distributions, particularly for <i>C. krusei</i> and for <i>C. auris</i> (17-21). For <i>C. auris</i>, a correlation between the Etest MIC and the inherent growth rate of the isolate has been shown (1, 22). 		

6. Monte Carlo simulations and PK/PD cut-off values

Aspergillus and *Candida*

Monte Carlo simulation analysis was performed for 5000 patients receiving L-AMB at the standard (3 mg/kg q24h i.v.), as well as the higher 5 mg/kg q24h i.v. dose, achieving blood levels corresponding to a mean±SD C_{max} 21.87±12.47 mg/L and 83±35.2 mg/L (Gilead Science Inc. AmBisome®), respectively and the *in vitro* PK/PD target C_{max}/MIC values of: 2.1 for *C. albicans*, 24 for *C. glabrata*, 8 for *C. parapsilosis*, 10 for *C. krusei* and 12 for *C. auris* were associated with stasis with the EUCAST method.



Beredaki et al Antimicrob Agents Chemother. 2024;68(8):e00225-24 (10), Beredaki et al J Infect Dis. 2024;229(2):599-607 (11)

7. Clinical data

Aspergillus

Amphotericin B was the cornerstone treatment for invasive aspergillosis for over 40 years. Whilst new treatment options have changed their role, lipid-associated amphotericin B regimens remain important therapeutic options for aspergillosis due to their broad-spectrum of activity and limited cross-resistance with triazole antifungals (23). Accurate speciation of *Aspergillus* species is important, as infections with some species, particularly *A. terreus*, *A. flavus*, and *A. nidulans* and potentially other respond poorly to amphotericin B therapy.

Amphotericin B is regarded first line antifungal therapy in patients with invasive aspergillosis resistant to voriconazole (23, 24). Amphotericin B formulations are alternative options for invasive aspergillosis, such as in patients who cannot tolerate voriconazole or with azole-resistant or refractory aspergillosis. Selecting the most appropriate lipid-based formulation of amphotericin remains a challenge and high-quality evidence from randomized, controlled trials is limited.

L-AmB vs D-AmB: In the study by Leenders et al. (25), L-AmB 5 mg/kg/day was compared with D-AmB 1 mg/kg/day. The patient population was severely neutropenic and had proven or probable invasive fungal infections; overall complete/partial responses with L-AmB were better than with D-AmB (50% vs 24%, $p=0.04$). Ellis et al. compared L-AmB 1 mg/kg/day with 4 mg/kg/day for efficacy in proven or probable invasive aspergillosis patients (26). There was no overall statistical significance between the survival rates at 6 months between the groups. Invasive aspergillosis was the primary cause of death for the same number of patients in both groups, but the group with definite invasive aspergillosis at the time of randomisation comprised only 20 patients, and their response rate was numerically higher on L-AmB 4 mg than on 1 mg/kg/day (58% versus 37%). In another study, L-AmB administered at a daily dose of 3 mg/kg was associated with similar efficacy, less nephrotoxicity, and a trend toward improved 12-week survival, as compared with a dose of 10 mg as primary therapy for invasive aspergillosis (72% vs 59% with a favourable response of 50% vs 46%, respectively) (27). This study showed that increased doses of amphotericin B should not be equated with greater efficacy.

ABLC vs D-AmB: Bowden et al. (28) compared ABLC 6 mg/kg/day with D-AmB 1-1.5 mg/kg/day against invasive aspergillosis in cancer patients and the results showed similar success rates for the two groups (52% versus 51%, respectively). An analysis of a large data registry on the use of ABLC in invasive aspergillosis showed encouraging findings regarding efficacy and safety, including the drug's tolerability in patients with renal impairment (29).

These studies did not include MICs by the EUCAST method so a correlation of *in vitro* MICs with clinical outcome has not been possible.

Candida

Amphotericin B was the cornerstone in the treatment of invasive *Candida* infections for many years. However, with the introduction of the echinocandin class of antifungals, amphotericin B is now regarded second line option for invasive candidiasis and first line option for resistant isolates (i.e. echinocandin-resistant *C. glabrata*) and for CNS infection (30). Clinical data for amphotericin B against *Candida* spp. was collated from the following clinical trials:

- Walsh et al. *NEJM*, 1999, 340:764-771 (31)
L-amphotericin B, 3mg/kg/d with 343 patients, mean treatment duration of 10.4 days. Showed a treatment success rate of 50.1%.
Amphotericin B deoxycholate, 0.6 mg/kg/d with 344 patients, mean treatment duration of 10.3 days. Showed a treatment success rate of 49.4%.
- Wingard et al. *CID* 2000, 31: 1155-1163 (32)
L-amphotericin B, 3mg/kg/d with 85 patients, mean treatment duration of 8.6 days. Showed a treatment success rate of 40%.
- Walsh et al. *NEJM* 2004,351: 1391-1402 (33)
L-amphotericin B, 3mg/kg/d with 539 patients, mean treatment duration of 12 days. Showed a treatment success rate of 33.7%.

- Walsh et al. *NEJM* 2002, 346: 225-234 (34)
L-amphotericin B, 3mg/kg/d with 422 patients, mean treatment duration of 12 days. Showed a treatment success rate of 30.6%.
- Kuse et al. *Lancet* 2007, 369: 1519-1527 (35)
L-amphotericin B, 3mg/kg/d with 190 patients, mean treatment duration of 15 days. Showed a treatment success of 89%
- Queiroz-Telles et al. *PIDJ* 2008, 27: 820-826 (36)
L-amphotericin B, 10 mg/kg/d with 50 patients, mean treatment duration of 14.5 days. Showed a treatment success rate of 76%.
- Fleming et al. *Leukemia and Lymphoma* 2001,40: 511-520 (37)
ABLCL, 5mg/kg/d with 70 patients (proven infections and empirical treatment), mean treatment duration of 12.4 days. Showed a treatment success rate of 63%.

For most of the studies, clinical outcome data was not provided for the individual *Candida* species. After combining the studies that provided such data (33, 35, 37), failure rates were as follows: For L-amphotericin B the overall failure rate was 9% (16/174) and for individual species: *C. albicans* 11% (7/73), *C. tropicalis* 4% (2/45), *C. parapsilosis* 10% (3/29), *C. glabrata* 20% (3/15) and *C. krusei* 20% (1/5). For amphotericin B deoxycholate the overall failure rate was 38% (44/115) and for individual species: *C. albicans* 8% and *C. krusei* 3%. These data indicate that the species are good targets for amphotericin B formulations.

C. auris. Clinical outcome data for amphotericin B against *C. auris* are sparse as echinocandins are recommended as 1st line agents for candidaemia in general and for *C. auris* specifically. CDC consider treatment with liposomal amphotericin B (5 mg/kg daily) indicated, when susceptibility testing suggests echinocandin resistance and in patients treated with echinocandins who do not improve after 5 days (<https://www.cdc.gov/candida-auris/hcp/clinical-care/index.html>). Several commercial AFST methods generate higher MICs for amphotericin B than the reference methods, which with a tentative resistance CDC BP of ≥ 2 mg/L (equals >1 mg/L in EUCAST terminology) resulted in high and variable resistance rates and reluctance to use the amphotericin B (19-21, 38). Moreover, the MICs are slightly ($\frac{1}{2}$ dilution) higher against *C. auris* than against *C. krusei*. In a recent systematic review, Sokou et al. (39) reported success for 2/2 neonates treated with amphotericin B deoxycholate versus 6/9 neonates treated with echinocandins and 8/14 treated with azoles, and for 5/5, 6/6 treated with combination therapy including amphotericin B and echinocandins and azoles respectively and 6/10 receiving azoles and echinocandin in combination. In a retrospective study in ICU patients with *C. auris* infections, treatment survival was similar between the echinocandin group (mainly anidulafungin) (7/12) compared to the liposomal amphotericin B 5 mg/kg group (4/5) (40).

Cryptococcus

Clinical data for amphotericin B against *Cryptococcus* spp.:

- Amphotericin B deoxycholate (in combination with flucytosine) is strongly recommended (has an A-I recommendation) for CNS cryptococcosis (41).
- Amphotericin B 1.0 mg/kg was associated with a significantly greater early fungicidal activity than amphotericin B 0.7 mg/kg when administered with flucytosine for induction therapy (42) (64 patients, AmB, 0.7 mg/kg per day, plus flucytosine, 25 mg/kg 4 times per day vs. AmB, 1 mg/kg per day, plus flucytosine, 25 mg/kg 4 times per day for 2 weeks, followed by treatment with oral fluconazole. Early fungicidal activity was significantly greater for group 2 than for group 1 (mean \pm SD, -0.56 ± 0.24 vs. -0.45 ± 0.16 log cfu/mL of cerebral spinal fluid per day; $P=0.02$) (35).
- L-amphotericin B was compared with conventional amphotericin B and found more effective in clearing the CSF and less nephrotoxic, but the time to and the rate of clinical response were the same in both arms (28 evaluable patients, L-amphotericin B 4 mg/kg daily vs. conventional amphotericin B 0.7 mg/kg daily for 3 weeks, each followed by fluconazole 400 mg daily for 7 weeks) (43).
- L-amphotericin B at 3 and 6 mg/kg was compared with conventional amphotericin B and found less toxic but with equal efficacy (267 patients, 11-21 days of study drug followed by fluconazole 400 mg until 10 weeks) (44).

Amphotericin B is the 1st line induction therapy for *C. neoformans* and *C. gattii* invasive infections, in combination with flucytosine when the CNS is involved. Acquired resistance to amphotericin B has not been reported and hence experience is absent regarding outcome for patients with isolates outside the wild-type population.

These studies did not include MICs by the EUCAST method. Therefore, correlating *in vitro* MICs with clinical outcome has not been possible.

8. Clinical breakpoints

	Organism group	MIC breakpoints (mg/L)		Notes
		S ≤	R >	
Species-related breakpoints	<i>A. fumigatus</i>	1	1	¹ Liposomal amphotericin B is recommended as 2 nd line option against <i>C. auris</i> infection, when echinocandins cannot be used, provided a high dose of 5 mg/kg/d is used. EUCAST supports a provisional cut-off value of ≤ 2 mg/L for this high dose although there are limited clinical data.
	<i>A. niger</i>	1	1	
	<i>C. albicans</i>	1	1	
	<i>C. auris</i>	0.001 ¹	2 ¹	
	<i>C. dubliniensis</i>	1	1	
	<i>C. glabrata</i>	1	1	
	<i>C. parapsilosis</i>	1	1	
	<i>C. tropicalis</i>	1	1	
	<i>C. krusei</i>	1	1	
	<i>Cryptococcus neoformans</i>	1	1	
Breakpoints were based on PK data, microbiological data, and patient outcomes from clinical trials.				
Species without breakpoints	<p>There is insufficient evidence to set clinical breakpoints for other species of <i>Aspergillus</i>.</p> <p><i>A. terreus</i>, <i>A. flavus</i>, and <i>A. nidulans</i> are not considered good targets for amphotericin B, which is therefore not recommended for treatment of invasive aspergillosis caused by these species. There are no data available regarding the underlying mechanisms of acquired resistance in <i>Aspergillus</i> isolates. Isolates with MICs higher than the breakpoint should be retested and send to a laboratory experienced in susceptibility testing of moulds.</p> <p>The clinical response of infection due to <i>Candida</i> species as a whole was similar to that of infections caused by <i>C. albicans</i>, <i>C. parapsilosis</i>, and <i>C. tropicalis</i> with the exception of <i>C. lusitaniae</i>. <i>C. lusitaniae</i> is a species that has been associated with increased risk of clinical failure to amphotericin B due to a higher mutational rate with occurrence of resistant mutant colonies upon exposure and less killing (45). Therefore, <i>C. lusitaniae</i> is not regarded a good target for amphotericin B and MIC testing for this organism is not recommended. For the remaining species, there were only 12 cases of infections due to less common <i>Candida</i> species available for analysis, which is too few to allow any recommendation to be made. Therefore, there is insufficient evidence to set clinical breakpoints for other species of <i>Candida</i>. Guidance for interpretation of MICs when there is no breakpoints can be found in the document: EUCAST guidance on Interpretation of MICs for rare yeast without breakpoints in breakpoint tables found at the link below (https://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals).</p>			

	<p>There are limited data available regarding the underlying mechanism of acquired amphotericin B resistance, but mutations in <i>ERG2</i>, <i>ERG3</i>, <i>ERG11</i> and <i>ERG5</i> have been reported in resistant clinical <i>Candida</i> isolates. Isolates with MICs higher than the breakpoint should be retested and send to a laboratory experienced in susceptibility testing of fungi.</p> <p>Amphotericin B in combination with flucytosine is the recommended treatment for <i>Cryptococcus gattii</i>. The MIC range appears similar to that of <i>Cryptococcus neoformans</i>, however there are insufficient data on MICs as well as clinical outcome for <i>Cryptococcus gattii</i> to set clinical breakpoints.</p>
Clinical qualifications	The EUCAST-AFST considers amphotericin B to be appropriate therapy for invasive aspergillosis, candidiasis, cryptococcosis, and mucormycosis.
Dosage	The EUCAST breakpoints apply to licensed dosing of amphotericin B deoxycholate, liposomal amphotericin B, and amphotericin B lipid complex. For <i>C. auris</i> , the breakpoint was set provided a high dose of liposomal amphotericin B of 5 mg/kg/d is used.
Additional comment	<p>There is no evidence to support routine therapeutic drug monitoring currently for amphotericin B (46).</p> <p>Antifungal susceptibility testing of amphotericin B against <i>C. auris</i> with several commercial methods (https://www.eucast.org/ast-of-bacteria/warnings) has been associated with overestimation of resistance due to greater variability and wider MIC distributions and/or higher MICs in several studies. (19-21, 38)</p> <p>The EUCAST-AFST will review breakpoints for amphotericin B when more data available for <i>Aspergillus</i> and <i>Candida</i> species which were not assigned breakpoints during the present review and when there are clinical data for <i>Aspergillus</i> and <i>Candida</i> isolates with MIC values outside the wild type distribution.</p>

References

1. Arendrup MC, Lockhart SR, Wiederhold N. *Candida auris* MIC testing by EUCAST and clinical and laboratory standards institute broth microdilution, and gradient diffusion strips; to be or not to be amphotericin B resistant? Clin Microbiol Infect. 2025;31(1):108-12.
2. Carolus H, Sofras D, Boccarella G, Sephton-Clark P, Biriukov V, Cauldron NC, et al. Acquired amphotericin B resistance leads to fitness trade-offs that can be mitigated by compensatory evolution in *Candida auris*. Nat Microbiol. 2024;9(12):3304-20.
3. Adedoyin A, Bernardo JF, Swenson CE, Bolsack LE, Horwith G, DeWit S, et al. Pharmacokinetic profile of ABELCET (amphotericin B lipid complex injection): combined experience from phase I and phase II studies. Antimicrob Agents Chemother. 1997;41(10):2201-8.
4. Bekersky I, Fielding RM, Dressler DE, Lee JW, Buell DN, Walsh TJ. Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate in humans. Antimicrob Agents Chemother. 2002;46(3):828-33.
5. Ayestaran A, Lopez RM, Montoro JB, Estibalez A, Pou L, Julia A, et al. Pharmacokinetics of conventional formulation versus fat emulsion formulation of amphotericin B in a group of patients with neutropenia. Antimicrob Agents Chemother. 1996;40(3):609-12.
6. Groll AH, Silling G, Young C, Schwerdtfeger R, Ostermann H, Heinz WJ, et al. Randomized comparison of safety and pharmacokinetics of caspofungin, liposomal amphotericin B, and the combination of both in allogeneic hematopoietic stem cell recipients. Antimicrob Agents Chemother. 2010;54(10):4143-9.
7. Walsh TJ, Goodman JL, Pappas P, Bekersky I, Buell DN, Roden M, et al. Safety, tolerance, and pharmacokinetics of high-dose liposomal amphotericin B (AmBisome) in patients infected with *Aspergillus* species and other filamentous fungi: maximum tolerated dose study. Antimicrob Agents Chemother. 2001;45(12):3487-96.
8. Andes D, Stamsted T, Conklin R. Pharmacodynamics of amphotericin B in a neutropenic-mouse disseminated-candidiasis model. Antimicrob Agents Chemother. 2001;45(3):922-6.
9. Andes D, Safdar N, Marchillo K, Conklin R. Pharmacokinetic-pharmacodynamic comparison of amphotericin B (AMB) and two lipid-associated AMB preparations, liposomal AMB and AMB lipid complex, in murine candidiasis models. Antimicrob Agents Chemother. 2006;50(2):674-84.
10. Beredaki MI, Arendrup MC, Pournaras S, Meletiadiis J. Comparative pharmacodynamics and dose optimization of liposomal amphotericin B against *Candida* species in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother. 2024;68(8):e0022524.
11. Beredaki MI, Sanidopoulos I, Pournaras S, Meletiadiis J. Defining optimal doses of liposomal amphotericin B against *Candida auris*: Data from an in vitro pharmacokinetic/pharmacodynamic model. J Infect Dis. 2024;229(2):599-607.
12. Seyedmousavi S, Melchers WJ, Mouton JW, Verweij PE. Pharmacodynamics and dose-response relationships of liposomal amphotericin B against different azole-resistant *Aspergillus fumigatus* isolates in a murine model of disseminated aspergillosis. Antimicrob Agents Chemother. 2013;57(4):1866-71.
13. Seyedmousavi S, Mouton JW, Melchers WJG, Verweij PE. In vivo efficacy of liposomal amphotericin B against wild-type and azole-resistant *Aspergillus fumigatus* isolates in two different immunosuppression models of invasive aspergillosis. Antimicrob Agents Chemother. 2017;61(6):e02479-16.
14. Mouton JW, te Dorsthorst DT, Meis JF, Verweij PE. Dose-response relationships of three amphotericin B formulations in a non-neutropenic murine model of invasive aspergillosis. Med Mycol. 2009;47(8):802-7.
15. Al-Nakeeb Z, Petraitis V, Goodwin J, Petraitiene R, Walsh TJ, Hope WW. Pharmacodynamics of amphotericin B deoxycholate, amphotericin B lipid complex, and liposomal amphotericin B against *Aspergillus fumigatus*. Antimicrob Agents Chemother. 2015;59(5):2735-45.
16. Lestner J, McEntee L, Johnson A, Livermore J, Whalley S, Schwartz J, et al. Experimental models of short courses of liposomal amphotericin B for induction therapy for cryptococcal meningitis. Antimicrob Agents Chemother. 2017;61(6):e00090-17.
17. Arendrup MC. *Candida* and candidaemia. Susceptibility and epidemiology. Dan Med J. 2013;60(11):B4698.
18. bioMérieux SA. Etest antifungal susceptibility testing package insert 15211G. bioMérieux SA, Marcy-l'Etoile, France. 2013.
19. Siopi M, Leventaki S, Pachoulis I, Pournaras S, Meletiadiis J, Spruijtenburg B, et al. Comparative evaluation of gradient concentration strip and reference CLSI methods for antifungal susceptibility testing of *Candida auris* using a representative international panel of isolates. ESCMID-Global2024.

20. Siopi M, Pachoulis I, Leventaki S, Spruijtenburg B, Meis JF, Pournaras S, et al. Evaluation of the Vitek 2 system for antifungal susceptibility testing of *Candida auris* using a representative international panel of clinical isolates: overestimation of amphotericin B resistance and underestimation of fluconazole resistance. *J Clin Microbiol*. 2024;62(4):e0152823.
21. Siopi M, Peroukidou I, Beredaki MI, Spruijtenburg B, de Groot T, Meis JF, et al. Overestimation of amphotericin B resistance in *Candida auris* with sensititre YeastOne antifungal susceptibility testing: a need for adjustment for correct interpretation. *Microbiol Spectr*. 2023;11(3):e0443122.
22. Arendrup MC, Prakash A, Meletiadiis J, Sharma C, Chowdhary A. Comparison of EUCAST and CLSI reference microdilution MICs of eight antifungal compounds for *Candida auris* and associated tentative epidemiological cutoff values. *Antimicrob Agents Chemother*. 2017;61(6):e00485-17.
23. Ullmann AJ, Aguado JM, Arikan-Akdagli S, Denning DW, Groll AH, Lagrou K, et al. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clin Microbiol Infect*. 2018;24 Suppl 1:e1-e38.
24. van der Linden JW, Camps SM, Kampinga GA, Arends JP, Debets-Ossenkopp YJ, Haas PJ, et al. Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. *Clin Infect Dis*. 2013;57(4):513-20.
25. Leenders AC, Daenen S, Jansen RL, Hop WC, Lowenberg B, Wijermans PW, et al. Liposomal amphotericin B compared with amphotericin B deoxycholate in the treatment of documented and suspected neutropenia-associated invasive fungal infections. *Br J Haematol*. 1998;103(1):205-12.
26. Ellis M, Spence D, de Pauw B, Meunier F, Marinus A, Collette L, et al. An EORTC international multicenter randomized trial (EORTC number 19923) comparing two dosages of liposomal amphotericin B for treatment of invasive aspergillosis. *Clin Infect Dis*. 1998;27(6):1406-12.
27. Cornely OA, Maertens J, Bresnik M, Ebrahimi R, Ullmann AJ, Bouza E, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis*. 2007;44(10):1289-97.
28. Bowden R, Chandrasekar P, White MH, Li X, Pietrelli L, Gurwith M, et al. A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients. *Clin Infect Dis*. 2002;35(4):359-66.
29. Chandrasekar PH, Ito JI. Amphotericin B lipid complex in the management of invasive aspergillosis in immunocompromised patients. *Clin Infect Dis*. 2005;40 Suppl 6:S392-400.
30. Lortholary O, Petrikkos G, Akova M, Arendrup MC, Arikan-Akdagli S, Bassetti M, et al. ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: patients with HIV infection or AIDS. *Clin Microbiol Infect*. 2012;18 Suppl 7:68-77.
31. Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, et al. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *N Engl J Med*. 1999;340(10):764-71.
32. Wingard JR, White MH, Anaissie E, Raffalli J, Goodman J, Arrieta A, Group LAACS. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. L Amph/ABLC Collaborative Study Group. *Clin Infect Dis*. 2000;31(5):1155-63.
33. Walsh TJ, Teppler H, Donowitz GR, Maertens JA, Baden LR, Dmoszynska A, et al. Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. *N Engl J Med*. 2004;351(14):1391-402.
34. Walsh TJ, Pappas P, Winston DJ, Lazarus HM, Petersen F, Raffalli J, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med*. 2002;346(4):225-34.
35. Kuse ER, Chetchotisakd P, da Cunha CA, Ruhnke M, Barrios C, Raghunadharao D, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. *Lancet*. 2007;369(9572):1519-27.
36. Queiroz-Telles F, Berezin E, Leverger G, Freire A, van der Vyver A, Chotpitayasunondh T, et al. Micafungin versus liposomal amphotericin B for pediatric patients with invasive candidiasis: substudy of a randomized double-blind trial. *Pediatr Infect Dis J*. 2008;27(9):820-6.
37. Fleming RV, Kantarjian HM, Husni R, Rolston K, Lim J, Raad I, et al. Comparison of amphotericin B lipid complex (ABLC) vs. amphotericin B in the treatment of suspected or documented fungal infections in patients with leukemia. *Leuk Lymphoma*. 2001;40(5-6):511-20.
38. Arendrup M, Lockhart S, Wiederhold N. *Candida auris* MIC testing by EUCAST, CLSI and strip tests; to be or not to be amphotericin B resistant? ESCMID-Global, 2024, Flash poster BES1109C.

39. Sokou R, Palioura AE, Kopanou Taliaka P, Konstantinidi A, Tsantes AG, Piovani D, et al. *Candida auris* infection, a rapidly emerging threat in the neonatal intensive care units: A systematic review. *J Clin Med*. 2024;13(6).
40. Paramythiotou E, Kyriazopoulou E, Karakike E, Siopi M, Frantzeskaki F, Meletiadis J, Tsangaris I. Clinical characteristics and outcome of *Candida auris* bloodstream infections during an outbreak in a Greek tertiary academic intensive care unit. *J Hosp Infect*. 2025;161:25-7.
41. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis*. 2010;50(3):291-322.
42. Bicanic T, Wood R, Meintjes G, Rebe K, Brouwer A, Loyse A, et al. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. *Clin Infect Dis*. 2008;47(1):123-30.
43. Leenders AC, Reiss P, Portegies P, Clezy K, Hop WC, Hoy J, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. *AIDS*. 1997;11(12):1463-71.
44. Hamill RJ, Sobel JD, El-Sadr W, Johnson PC, Graybill JR, Javaly K, Barker DE. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis*. 2010;51(2):225-32.
45. Atkinson BJ, Lewis RE, Kontoyiannis DP. *Candida lusitanae* fungemia in cancer patients: risk factors for amphotericin B failure and outcome. *Med Mycol*. 2008;46(6):541-6.
46. Ashbee HR, Barnes RA, Johnson EM, Richardson MD, Gorton R, Hope WW. Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology. *J Antimicrob Chemother*. 2014;69(5):1162-76.