EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Rationale for EUCAST clinical breakpoints

Agent	**Amphotericin B**

Current version	**2.0**	**4th of February 2020 (Amphotericin B)**
Previous versions	1.0	19th November 2010 (Amphotericin B vs *Candida* spp.)
	1.0	15th July 2011 (Amphotericin B vs *Aspergillus* 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.

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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 *Aspergillus flavus, Aspergillus terreus*, *Aspergillus nidulans*, *Aspergillus lentulus* and *Aspergillus 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 parapsilosis, Candida tropicalis, Candida glabrata* and *Candida krusei.* The *in vitro* activity of amphotericin B against species of *Candida* is mostly uniform. Amphotericin B has limited clinical activity against *Candida lusitaniae* 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.

In version 2.0 of this rationale document, the *Aspergillus* and *Candida* rational documents have been combined into a single document, ECOFFs for *Cryptococcus neoformans* have been included, and MIC distribution tables updated with more information on *Candida* spp., *Aspergillus* spp., and *Cryptococcus neoformans*. The information on the ABCD formulation has been removed as it is no longer available. Last but not least the R breakpoint has been lowered for all species to adopt the breakpoints to the revised definition of the I category (Susceptible, Increased exposure) because no evidence exist that dose escalation is a valid option for isolates in the previous Intermediate category.

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
1st 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
2nd 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*

	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
1st day dose (mg/kg/day)	3	1	3	1	1	1	1	1	1	3	1
2nd 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
1st day dose (mg/kg/day)		5	5			5	NA	NA	3-5.5	5	5
2nd 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)

	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	ECOFF/ [T-ECOFF]*
Aspergillus flavus	0	0	0	0	0	0	0	6	42	83	59	15	1	1	0	0	0	0	0	4
Aspergillus fumigatus	0	0	0	1	1	11	384	593	1216	223	12	1	0	0	0	0	0	0	0	1
Aspergillus nidulans	0	0	0	0	0	2	4	8	37	38	20	4	3	5	0	0	0	0	0	[4] [a]
Aspergillus niger	0	0	0	1	6	27	50	70	28	2	0	1	0	0	0	0	0	0	0	[0.5]
Aspergillus terreus	0	0	0	0	0	0	0	3	11	42	69	48	12	3	0	0	0	0	0	8
Fusarium (Gibberella) fujikuroi SC [b]	0	0	0	0	0	0	2	0	2	13	41	29	11	1	0	0	0	0	0	[8]
Fusarium solani SC	0	0	1	0	0	0	0	0	5	33	19	6	7	1	0	0	0	0	0	[8]
Candida albicans	0	0	1	2	13	59	518	506	223	19	1	0	0	0	0	0	0	0	0	1
Candida dubliniensis	0	0	0	2	41	61	26	10	6	0	0	0	0	0	0	0	0	0	0	0.25
Candida glabrata	0	0	0	0	9	13	215	417	230	22	1	0	0	0	0	0	0	0	0	1
Candida guilliermondii	0	0	0	0	8	21	38	17	4	0	0	0	0	0	0	0	0	0	0	[0.5]
Candida krusei	0	0	0	0	0	0	6	35	167	53	1	0	0	0	0	0	0	0	0	1
Candida parapsilosis	0	0	0	0	5	8	26	116	141	17	0	0	0	0	1	0	0	0	0	1
Candida tropicalis	0	0	0	0	0	4	31	134	81	7	0	0	0	0	0	0	0	0	0	1
Candida kefyr	0	0	0	0	0	1	11	19	26	7	0	0	0	0	0	0	0	0	0	[1]
Candida lusitaniae	0	0	0	0	2	21	17	14	5	0	0	0	0	0	0	0	0	0	0	[0.5]
Saccharomyces cerevisiae	0	0	0	1	2	9	21	37	10	1	0	0	0	0	0	0	0	0	0	[0.5]
Cryptococcus neoformans	0	0	0	1	12	82	371	406	123	25	2	0	0	0	0	0	0	0	0	[1]
Cryptococcus gattii	0	0	0	0	2	11	25	13	1	0	0	0	0	0	0	0	0	0	0	[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. When there is insufficient evidence, no ECOFFs have been determined (ND).

* tentative T-ECOFF was determined because <5 qualified MIC distributions were aggregated, and is indicated in brackets.

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

[b] SC: species complex

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

	No.	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	ECOFF/ [T-ECOFF]*
Aspergillus flavus	207	0	0	0	0	0	0	0	3	20	40	29	7	0	0	0	0	0	0	0	4
Aspergillus fumigatus	2627	0	0	0	0	0	0	15	23	46	8	0	0	0	0	0	0	0	0	0	1
Aspergillus nidulans	121	0	0	0	0	0	2	3	7	31	31	17	3	2	4	0	0	0	0	0	[4] [a]
Aspergillus niger	185	0	0	0	1	3	15	27	38	15	1	0	1	0	0	0	0	0	0	0	[0.5]
Aspergillus terreus	188	0	0	0	0	0	0	0	2	6	22	37	26	6	2	0	0	0	0	0	8
Fusarium (Gibberella) fujikuroi SC[b]	99	0	0	0	0	0	0	2	0	2	13	41	29	11	1	0	0	0	0	0	[8]
Fusarium solani SC	72	0	0	1	0	0	0	0	0	7	46	26	8	10	1	0	0	0	0	0	[8]
Candida albicans	1342	0	0	0	0	1	4	39	38	17	1	0	0	0	0	0	0	0	0	0	1
Candida dubliniensis	146	0	0	0	1	28	42	18	7	4	0	0	0	0	0	0	0	0	0	0	0.25
Candida glabrata	907	0	0	0	0	1	1	24	46	25	2	0	0	0	0	0	0	0	0	0	1
Candida guilliermondii	88	0	0	0	0	9	24	43	19	5	0	0	0	0	0	0	0	0	0	0	[0.5]
Candida krusei	262	0	0	0	0	0	0	2	13	64	20	0	0	0	0	0	0	0	0	0	1
Candida parapsilosis	314	0	0	0	0	2	3	8	37	45	5	0	0	0	0	0	0	0	0	0	1
Candida tropicalis	257	0	0	0	0	0	2	12	52	32	3	0	0	0	0	0	0	0	0	0	1
Candida kefyr	64	0	0	0	0	0	2	17	30	41	11	0	0	0	0	0	0	0	0	0	[1]
Candida lusitaniae	59	0	0	0	0	3	36	29	24	8	0	0	0	0	0	0	0	0	0	0	[0.5]
Saccharomyces cerevisiae	81	0	0	0	1	2	11	26	46	12	1	0	0	0	0	0	0	0	0	0	[0.5]
Cryptococcus neoformans	1022	0	0	0	0	1	8	36	40	12	2	0	0	0	0	0	0	0	0	0	[1]
Cryptococcus gattii	52	0	0	0	0	4	21	48	25	2	0	0	0	0	0	0	0	0	0	0	[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. When there is insufficient evidence, no ECOFFs have been determined (ND).

* tentative T-ECOFF was determined because <5 qualified MIC distributions were aggregated, and is indicated in brackets.

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

[b] SC: species complex

4. Breakpoints prior to harmonisation (mg/L) S≤ R>

	European breakpoints	CLSI
General breakpoints:		
	NA	NA
Species specific breakpoints:		
	NA	NA[1]

NA = Not available
[1]No breakpoint has been established for amphotericin B, but, according to the text in CLSI M27-A3, *Candida* isolates with MIC ≤1 mg/L should be regarded as susceptible.

5. Pharmacokinetics

	Amphotericin B deoxycholate			Amphotericin B lipid complex		Liposomal amphotericin B[2]		
Patients group, Reference	HIV patients, (1)	Healthy, (2)	Healthy, (3)	HIV patients, (1)	Immunocom-promised, (4)	Allo-HSCT, (5)	Immunocompromised, (6)	
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 ½ (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
$AUC_{0\text{-}24}$h (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, Vc (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 (2). • [2]Pharmacokinetics may vary with dosages >7.5 mg/kg • Cells are left empty when data are not readily available							

6. Pharmacodynamics

	***Aspergillus* spp.**	***Candida* spp.**	***Cryptococcus* spp.**
Fungistatic/1-log kill dose (mg/kg) for fungal burden in kidney in neutropenic murine model of disseminated candidiasis (7)		*C. albicans* (N=3) (MIC= 0.25 mg/L) D-AmB= 1.86-4.46/2.70->20 *C. krusei* (N=1) (MIC= 0.25 mg/L) D-AmB= 1.94/8.01 *C. dubliniensis* (N=1) (MIC= 0.5 mg/L) D-AmB= 3.60/>20	
ED_{50}/1-logkill dose (mg/kg) for fungal burden in kidney in neutropenic murine model of disseminated candidiasis (8)		*C. albicans* (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	
ED_{50}/ED_{80} dose (mg/kg) for 14d survival in a murine non-neutropenic model of disseminated aspergillosis (9)	*A. fumigatus* (N=4) (MIC=0.5 mg/L) L-AmB= 0.08-0.60/1.26-17.4		
ED_{50} dose (mg/kg) for 14d survival in a murine neutropenic/immunosuppressed model of disseminated aspergillosis (10)	*A. fumigatus* (N=2) (MIC=0.5 mg/l) L-AmB= 1.4-1.92/2.40-2.56		
ED_{50} dose (mg/kg) for 15d survival in a murine non-neutropenic murine model of disseminated aspergillosis (11)	*A. fumigatus* (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 (12)	*A. fumigatus* (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 (13)			*C. neoformans* var. *grubii* (N=1) L-A-B= ~100
Comments	• Cells are left empty when data are not readily available.		

7. Monte Carlo simulations and Pk/Pd breakpoints

Aspergillus and *Candida*

The PK/PD relationship of different AMB forms is not well understood and the link between serum concentrations of different AMB forms with the efficacy is not well defined.

8. Clinical data

Aspergillus

Polyene antifungals were 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 (14). Recent studies have demonstrated the importance of accurate speciation of *Aspergillus* species during amphotericin B therapy, 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 (14, 15). Amphotericin B formulations are alternative options for invasive aspergillosis, such as in patients who cannot tolerate voriconazole or with 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. (16) 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 (17). 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) (18); this study showed that increased doses of amphotericin B should not be equated with greater efficacy.

ABLC vs D-AmB: Bowden et al. (19) 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 invasive aspergillosis showed encouraging findings regarding efficacy and safety, including the drug's tolerability in patients with renal impairment (20).

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 (21). Clinical data for amphotericin B against *Candida* spp. was collated from the following clinical trials:

- Walsh et al. *NEJM,* 1999, 340:764-771 (22)
  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 (23)
  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 (24)
  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 (25)
  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 (26)
  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 (27)
  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 (28)
  ABLC, 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 (24, 26, 28), 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.

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 (29).
- 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 (30) (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 ± 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) (31).
- 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) (32).

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 so a correlation of *in vitro* MICs with clinical outcome has not been possible.

9. *Aspergillus*, *Candida*, and *Cryptococcus* Clinical breakpoints

Non-species-related breakpoints

Breakpoints were based on pharmacokinetic data, microbiological data and clinical experience.

For *Aspergillus*, clinical experience suggest that species with wild type MICs at and below 1 mg/L are appropriate targets for amphotericin B. EUCAST therefore regard S ≤1, R >1 mg/L as a tentative breakpoint for species not assigned species specific breakpoints.

For *Candida*, the clinical experience over the past decades has not suggested that clinical resistance is a common feature in absence of MIC elevation above 1 mg/L except in *C. lusitaniae* as detailed below. Therefore, EUCAST has set a non-species specific breakpoint of S: ≤1 mg/L, R >1 mg/L for *Candida* spp. except *C. lusitaniae*.

Species-related breakpoints

Organism group	MIC breakpoints (mg/L)		Notes
	S ≤	R >	
A. fumigatus	1	1	
A. niger	1	1	
C. albicans	1	1	
C. dubliniensis	1	1	
C. glabrata	1	1	
C. parapsilosis	1	1	
C. tropicalis	1	1	
C. krusei	1	1	
Cryptococcus neoformans	1	1	

Breakpoints were based on PK data, microbiological data, and patient outcomes from clinical trials.

Species without breakpoints

There is insufficient evidence to set clinical breakpoints for other species of *Aspergillus.*

A. terreus, A. flavus, and *A. nidulans* 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 *Aspergillus* isolates. Isolates with MICs higher than the breakpoint should be retested and send to a laboratory experienced in susceptibility testing of moulds.

The clinical response of infection due to *Candida* species as a whole was similar to that of infections caused by *C. albicans, C. parapsilosis,* and *C. tropicalis* with the exception of *C. lusitaniae. C. lusitaniae* 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 (33). Therefore, *C. lusitaniae* 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 *Candida* 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 *Candida*. There are limited data available regarding the underlying mechanism of acquired amphotericin B resistance, but mutations in *ERG2, ERG3, ERG11* and *ERG5* have been reported in resistant clinical *Candida* isolates. Isolates with MICs higher than the breakpoint should be retested and send to a laboratory experienced in susceptibility testing of fungi. Amphotericin B in combination with flucytosine is the recommended treatment for *Cryptococcus gattii* also. The MIC range appears similar to that of *Cryptococcus neoformans*, however there are sparse data on MICs as well as clinical outcome for *Cryptococcus gattii* to set clinical breakpoints
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.
Additional comment	There is no evidence to support routine therapeutic drug monitoring currently for amphotericin B (34). The EUCAST-AFST will review breakpoints for amphotericin B when more data available for *Aspergillus* and Candida species which were not assigned breakpoints during the present review and when there are clinical data for *Aspergillus* and *Candida* isolates with MIC values outside the wild type distribution.

10. Exceptions noted for individual national committees

None

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