Evaluation of the in vitro activity of Amphotericin B used in the liposomal formulation AmBisome against contemporary human Fungal pathogens

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Objective. Amphotericin B (AmB) is the antifungal drugs that present most potent activity and wider spectrum of action, but its use has been classically limited by its secondary effects. To reduce the toxicity, nowadays it is mainly administered as liposomal formulation, mainly AmBisome (Gilead). Paradoxically, a full description of the activity of the AmB used in this formulation has not been reported. The objective of this study is to describe the in vitro antifungal activity of AmB used in the AmBisome formulation.

Methods. We carried out a multicenter study to characterize the antifugal susceptibility to AmB from AmBisome (provided by Gilead, G-AmB). We used two standardized methods, CLSI and EUCAST. We evaluated this activity against the most prevalent fungi in clinic (including both yeasts and filamentous fungi). Representative isolates from different geographical regions were included. Other antifungals were evaluated (fluconazole, itraconazole, voriconazole, posaconazole, caspofungin, micafungin and anidulafungin).

RESULTS

We sumarize the susceptibility of yeast and filamentous fungi to G-AmB and other antifungals. The number of isolates (N) and Geometric Mean (GM, mg/L) of the MIC values is shown. In total we obtain around 4,000 MICs. Data of CLSI and EUCAST have been grouped.

Species		G-AmB	Fluconazole	Voriconazole	Caspofungin	Micafungin	Anidulafungin
Candida albicans	N	196	196	196	196	196	196
	GM (mg/L)	0.24	0.34	0.03	0.13	0.02	0.03
Candida parapsilosis	N	206	206	206	206	206	206
	GM (mg/L)	0.32	0.78	0.03	0.65	0.82	1
Candida glabrata	N	229	229	229	229	229	229
	GM (mg/L)	0.35	10.10	0.31	0.23	0.02	0.04
Candida tropicalis	N	180	180	180	180	180	180
	GM (mg/L)	0.34	2.14	0.21	0.16	0.03	0.03
Candida krusei	N	171	171	171	171	171	171
	GM (mg/L)	0.56	37.63	0.28	0.39	0.12	0.06
Candida dubliniensis	N	78	78	78	78	78	78
	GM (mg/L)	0.15	0.23	0.02	0.37	0.06	0.08
Candida auris	N	42	42	42	42	42	42
	GM (mg/L)	0.37	>64	1.08	0.66	0.05	0.04
Cryptococcus neoformans	N	141	141	141	141	141	141
	GM (mg/L)	0.22	5.87	0.09	15.9	>8	>8
Candida kefyr	N	39	39	39	39	39	39
	GM (mg/L)	0.33	0.51	0.03	0.17	0.06	0.09
Candida lusitaniae	N	46	46	46	46	46	46
	GM (mg/L)	0.27	0.54	0.03	0.46	0.10	0.14
Saccharomyces cerevisiae	N	40	40	40	40	40	40
	GM (mg/L)	0.32	2.07	0.05	0.32	0.11	0.13
Candida guilliermondii	N	73	73	73	73	73	73
	GM (mg/L)	0.10	2.51	0.09	0.40	0.17	0.81
Rhodotorula rubra	N	6	6	6	6	6	6
	GM (mg/L)	0.18	>64	1.78	5.66	11.31	11.31
Trichosporum ashaii	N	50	20	50	35	35	35
	GM (mg/L)	2.14	21.86	1.60	>16	>8	>8
Candida inconspicua	N	8	8	8	8	8	8
	GM (mg/L)	0.16	8.72	0.11	0.35	0.09	0.23
Cryptococcus gatii	N	32	32	32	32	32	32
	GM (mg/L)	0.16	14.99	0.30	16	>8	>8
Candida famata	N	5	5	5	5	5	5
	GM (mg/L)	0.25	1.15	0.03	0.44	0.57	1.00
Rhodotorula mucilaginosa	N	20	20	20	20	20	20
	GM (mg/L)	2.55	14.93	0.45	>16	11.31	8.00

G-AmB shows a strong activity against most yeasts using both protocols (EUCAST and CLSI), in particular *C. albicans*, *C. parapsilosis* and *C. glabrata*. It also has a strong activity against *C. auris* (which was fully resistant to fluconazole) and *C. neoformans* (resistant to echinocandins). The least susceptible yeasts were Trichosporon and *Rhodotorula* (GM around 2.5 mg/L).

Species		G-AmB	Itraconazole	Voriconazole	Posaconazole
Aspergillus fumigatus	N	283	282	283	283
	GM (mg/L)	0.56	0.38	0.54	0.15
Aspergillus flavus	N	191	191	191	191
	GM (mg/L)	0.90	0.21	0.48	0.11
Aspergillus terreus	N	164	164	164	164
	GM (mg/L)	1.25	0.14	0.49	0.09
Aspergillus nidulans	N	82	82	82	82
	GM (mg/L)	0.96	0.20	0.18	0.09
Aspergillus niger	N	205	203	205	205
	GM (mg/L)	0.27	0.78	0.72	0.18
Rhizopus arrhizus	N	102	102	102	102
	GM (mg/L)	0.28	1.41	6.94	0.50
Lichtheimia corymbifera	N	112	112	112	112
	GM (mg/L)	0.14	0.42	16.10	0.33
Mucor circinelloides	N	72	72	72	72
	GM (mg/L)	0.11	8.08	22.41	4.16
Rhizomuchor pusillus	N	60	60	60	60
	GM (mg/L)	0.11	0.44	15.45	0.37
Lichtheimia ramosa	N	70	70	70	70
	GM (mg/L)	0.10	0.32	14.78	0.27
Rhizopus microsporus	N	110	110	110	110
	GM (mg/L)	0.32	1.51	5.34	0.57
Exophiala dermatitidis	N	68	68	68	68
	GM (mg/L)	0.30	0.15	0.19	0.09

Species		G-AmB	Itraconazole	Voriconazole	Posaconazole
Fusarium oxysporum	N	96	96	96	96
	GM (mg/L)	1.37	18.09	3.51	5.57
Fusarium solani	N	90	90	90	90
	GM (mg/L)	1.27	16.75	11.94	21.43
Lomentospora prolificans	N	138	137	138	137
	GM (mg/L)	13.97	19.79	17.87	23.27
Cunninghamella bertholletiae	N	38	38	38	38
	GM (mg/L)	4.46	2.04	17.21	0.98
Fusarium proliferatum	N	20	20	20	20
	GM (mg/L)	1.62	22.63	9.51	14.93
Scedosporium apiospermum	N	158	158	158	158
	GM (mg/L)	3.16	8.85	0.81	1.96
Talaromyces marneffei	N	40	40	40	40
	GM (mg/L)	0.14	0.02	0.03	0.03
Aspergillus sydowii	N	26	26	26	26
	GM (mg/L)	2.54	1.57	1.05	0.41
Fusarium verticilloides	N	20	20	20	20
	GM (mg/L)	1.57	13.93	7.21	10.2
Paecilomyces variotii	N	6	6	6	6
	GM (mg/L)	0.06	0.11	0.79	0.07
Aspergillus versicolor	N	8	8	8	8
	GM (mg/L)	0.18	0.08	0.27	0.07
Aspergillus welwitschiae	N	36	36	36	36
	GM (mg/L)	0.43	0.62	0.46	0.24
Scedosporium boydii	N	86	86	86	86
	GM (mg/L)	5.26	4.93	0.68	1.07
Scedosporium aurantiacum	N	60	60	60	60
	GM (mg/L)	11.99	6.73	0.97	0.55

The activity of G-AmB against filamentous fungi strongly depends on the species. *Aspergillus fumigatus* and *A. niger* are the most susceptible molds(GM 0.3-0.5 mg/L) . MICs to G-AmB were higher for the other *Aspergillus* spp, (geometric mean around 1-2 mg/L). G-AmB also showed a strong activity against other fungi that showed reduced susceptibility to other antifungals, such as *F. solani* and *F. oxysporum, Mucor* and *Rhizopus.* In contrast, G-AmB had limited activity against some multiresistant fungi, such as *Lomentospora* and *Scedosporium*

CONCLUSIONS: This is the largest interlaboratory study that describes the activity of the AmB formulation used to prepare the liposomal formulation AmBisome using two CLSI and EUCAST protocols. G-AmB shows a strong activity against most of the pathogenic fungal species (*Candida, Cryptococcus and Aspergillus spp*). We did not find significant differences between the susceptibility methods. This work has provided the most detailed description of the antifungal activity of the AmB used to prepare the liposomal formulation AmBisome and that is widely used in clinic, compared to other formulations that present higher toxicity.

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