Novel Rapid Antifungal Susceptibility Testing System for Yeasts Based on Fast Cell Growth

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Background

The traditional methods of antifungal susceptibility testing (AFST) have turnaround times of 24 to 48 h, rapid AFST is urgently needed in clinics to treat invasive fungal infections with the appropriate antifungal drugs and to slow the emergence of antifungal resistance.

Methods

The rapid AFST system follows the same operation procedure of a traditional AFST as shown in Figs 1 and 2. A total of 32 randomly selected clinical fungal strains were analyzed at the Peking Union Medical College Hospital. The performance of this rapid AFST method in terms of minimum inhibitory concentration (MIC) for isavuconazole was compared with the standard broth microdilution method according to CLSI M27 Ed4 guidelines. The comparator method for the remaining nine antifungal agents was the Sensititre YeastOne colorimetric panel. For the rapid AFST method, MICs were determined at 5-7 h of incubation, with extended incubation to 12 h for *Candida glabrata*. MICs for broth microdilution and the YeastOne panel were determined at 24 to 48 h of incubation.

Results

Table 1 shows summary results for ten antifungal agents in terms of essential agreement (EA) between the rapid AFST and the reference/comparator method. For AFST EA is achieved if the MIC obtained with the test method is within 2 two-fold dilution of that obtained with the reference or comparator method according to the CLSI guideline M52. EA is more than 93% for all 10 drugs. Table 2 shows MIC values for quality controls and typical clinical strains between the rapid AFST and reference/comparator methods.

Conclusions

The performance of this reported rapid AFST system is substantially equivalent to that of the standard microdilution method and the Sensititre YeastOne method. The rapid AFST system can be an ideal alternative AFST method given that it reduces the turnaround time significantly.

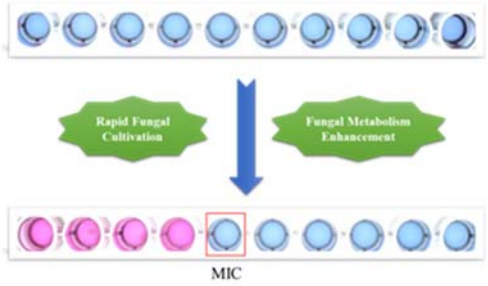

Fig 1 Detection principle of the rapid AFST.

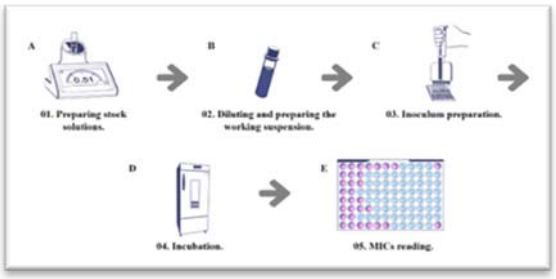

Fig 2 Operation procedure of the rapid AFST.

Table 1 Summary of EA between reference method and the rapid AFST for *Candida* species.

Antifungal agents	Reportable Range(μg/ml)		N_{EA}	N_T	EA $[N_{EA}/ N_T]$
	Reference method	Rapid AFST			
Fluconazole	0.12-256	0.12-64	31	32	96.88%
Itraconazole	0.016-16	0.03-16	30	32	93.75%
Voriconazole	0.008-8	0.016-8	32	32	100.00%
Posaconazole	0.008-8	0.03-16	30	32	93.75%
Isavuconazole	0.03-16	0.016-8	30	32	93.75%
Caspofungin	0.008-8	0.016-8	31	32	96.88%
Micafungin	0.008-8	0.016-8	30	32	93.75%
Anidulafungin	0.016-8	0.03-4	31	32	96.88%
5-flucytosine	0.06-64	0.12-64	32	32	100.00%
Amphotericin B	0.12-8	0.12-8	32	32	100.00%

Abbreviations: NEA, number of tests that resulted in EA; NT, total number of clinical isolates tested.

Table 2 MIC values obtained by reference methods and rapid AFST for quality control strains and typical strains of clinical isolates

Antifungal agents	Quality control strains				Clinical strains													
	Candida parapsilosis ATCC 22019		*Candida krusei* ATCC 6258		*Candida albicans* (n=9)		*Candida krusei* (n=5)		*Candida tropicalis* (n=3)		*Candida parapsilosis* (n=3)		*Candida lusitaniae* (n=3)		*Candida glabrata* (n=7)		*Candida guilliermondii* (n=2)	
	QC Range	Rapid AFST	QC Range	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST	Reference method	Rapid AFST
Fluconazole	0.5-4	4	8-64	16	1	1	32	16	2	1	0.25	0.5	0.5	1	32	64	>256	>64
Itraconazole	0.06-0.5	0.25	0.12-1	0.25	0.06	0.12	0.25	0.25	0.25	0.25	≤0.016	0.06	0.06	0.12	0.12	0.25	>16	>16
Voriconazole	0.016-0.12	0.06	0.06-0.5	0.12	0.016	0.016	0.25	0.12	0.12	0.12	≤0.008	≤0.016	≤0.008	≤0.016	0.25	1	>8	8
Posaconazole	0.03-0.25	0.12	0.06-0.5	0.12	0.016	0.016	0.25	0.25	0.12	0.25	0.015	0.03	0.016	≤0.03	1	0.5	1	2
Isavuconazole	0.016-0.06	0.03	0.06-0.5	0.06	0.03	0.03	0.06	0.12	0.12	0.12	≤0.03	≤0.016	0.03	0.016	0.03	0.016	4	4
Caspofungin	0.25-1	0.5	0.12-1	0.12	0.03	0.12	0.5	0.12	0.06	0.03	0.5	0.25	0.12	0.06	0.06	0.06	0.12	0.06
Micafungin	0.5-2	1	0.06-0.25	0.06	≤0.008	≤0.016	0.06	0.06	0.03	0.03	1	0.5	0.06	0.03	0.016	≤0.016	0.12	0.12
Anidulafungin	0.25-2	0.5	0.03-0.12	0.03	≤0.016	≤0.03	0.03	0.03	0.25	0.12	1	0.25	0.12	0.03	0.03	0.03	0.5	0.25
5-flucytosine	0.06-0.25	0.25	4-16	4	≤0.06	≤0.12	8	4	≤0.06	≤0.12	≤0.06	≤0.12	≤0.06	≤0.12	≤0.06	≤0.12	>64	>64
Amphotericin B	0.25-2	0.5	0.5-2	0.5	0.5	0.25	0.5	0.5	1	0.25	0.5	0.25	0.5	0.25	0.5	0.5	0.5	0.25