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EDITORIAL

Antifungal susceptibility testing in Australasian clinical laboratories: we must improve our performance

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Many Australasian microbiology laboratories are in the process of or have already transitioned from Clinical Laboratory Standards Institute (CLSI)-based antibiotic susceptibility testing to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) based method. This move has occurred in part because of the robust approach taken by EUCAST to set minimum inhibitory concentration (MIC) clinical breakpoints (CBPs), including basing CBPs on (1) drug pharmacokinetic/pharmacodynamic characteristics, and (2) drug resistance mechanisms, wherever possible.[1] The EUCAST steering committee is responsive to the changing epidemiology of antimicrobial resistance with attendant need for regular review of existing CBPs as well as antimicrobials that urgently need CBPs set. Changes in methodology and interpretive parameters are published regularly and are available at no cost through the EUCAST website (http://www.eucast.org/). By comparison, CLSI updates tend to be less frequent and are available at a cost (https://clsi.org/).

Whilst this changeover has been straightforward in the context of antibacterial susceptibility testing, it has led to some confusion on how to deal with antifungal susceptibility testing (AFST). At present, there are (to our knowledge) no diagnostic laboratories in Australia or New Zealand that follow the EUCAST standard for yeast or mould AFST, nor are there comparable EUCAST-based commercial methods available. Therefore, for pragmatic reasons many laboratories are using EUCAST-based methods for antibacterial testing and CLSI-based methods for AFST. However, problems arise if laboratories apply EUCAST antifungal interpretive criteria to their CLSI-based methodologies.

AFST results are influenced by methodological factors, including: (1) isolate growth phase and inoculum size; (2) incubation time and temperature; (3) media type, particularly relating to glucose concentration; (4) purity, concentration and activity of the antifungal agent; (5) endpoint stringency (e.g., 50%, 90%, or 100% growth inhibition); and (6) whether these endpoints are read visually or spectrophotometrically.[2] This wide range of variables formed the rationale for developing methodology standards by CLSI and EUCAST.[3–6] Each method is time tested, accurate and reliable, but labour-intensive and requires significant training of staff, maintenance of expertise, and a large volume of isolates to be cost effective. Due to methodological differences, CLSI and EUCAST standard methodologies often yield different MICs and therefore must only be interpreted using the applicable breakpoints. Table 1 shows the current CBPs and epidemiological cut-off values (ECVs) available to interpret applicable drug/species.[7–9] Some of these breakpoints differ between CLSI and EUCAST by as much as 3 dilutions. Therefore, using EUCAST interpretive criteria for CLSI generated MICs (or vice versa) could lead to misclassification of 'susceptible' isolates as 'resistant', or 'resistant' isolates as 'susceptible', particularly where an MIC is close to the breakpoint. Such false-susceptible or false-resistant errors may lead to erroneous clinical advice. Clearly there are gaps in the coverage of breakpoints and interpretive criteria by both CLSI and EUCAST, but the temptation to 'mix and match' CLSI and EUCAST criteria must be resisted.

Current workload pressures in laboratories, coupled with a structure of rotating non-specialised staff, do not align with routine use of either of these reference AFST methods. Instead the use of commercial tests such as the Sensititre YeastOne (SYO) (Trek Diagnostics, USA) offers many practical advantages. This 'CLSI-compatible' colorimetric broth microdilution method shares fundamental aspects of its procedure with the CLSI standard and has well established performance equivalence with the CLSI standard.[10,11] This method must only be used in association with current CLSI-endorsed CBPs and ECVs.[8,9]

The Micronaut-AM EUCAST AFST (Merlin-Diagnostika, Germany) is a commercially available EUCAST-based colorimetric broth microdilution method for six antifungal agents, however there are no published studies comparing its performance with the EUCAST standard. To our knowledge there are no Australasian laboratories using this method and it has not been extensively evaluated.

The Vitek 2 automated system (bioMérieux, France) provides yeast identification and AFST results in 14–18 h. Seven antifungal agents are available for testing, however only fluconazole, caspofungin and voriconazole are approved by the US Food and Drug Administration.[11] Studies have reported reproducible and accurate results between the Vitek 2 and CLSI/EUCAST reference methods with essential agreements of 88–98%.[10,12–14] However, a limitation of Vitek 2 AST is that the lowest caspofungin concentration (0.25 mg/L) is higher than the *Candida glabrata* CLSI

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Table 1 Current clinical breakpoints (CBPs) and epidemiological cut-off (ECV or ECOFF) values based on MICs or MECs for *Candida* and *Aspergillus* species as endorsed by CLSI and EUCAST[7–9]

Antifungal agent	Species	Species-specific interpretive categories for CLSI vs EUCAST 2-fold broth microdilution MICs (mg/L)								
		S≤		I=		SDD=	R≥		WT≤	NWT≥
		CLSI	EUCAST	CLSI	EUCAST	CLSI	CLSI	EUCAST	CLSI	CLSI
ANID	*C. albicans*	**0.25**	**0.03**	0.5			**1**	**0.06**		
	C. dubliniensis								0.12	0.25
	C. glabrata	0.12	0.06	0.25			**0.5**	**0.12**		
	C. guilliermondii	2		4			8			
	C. krusei	**0.25**	**0.06**	0.5			**1**	**0.12**		
	C. lusitaniae								1	2
	C. parapsilosis	**2**	**0.002**	4	0.004–4		8	8		
	C. tropicalis	**0.25**	**0.06**	0.5			**1**	**0.12**		
CASP	*C. albicans*	0.25		0.5			1			
	C. glabrata	0.12		0.25			0.5			
	C. guilliermondii	2		4			8			
	C. krusei	0.25		0.5			1			
	C. parapsilosis	2		4			8			
	C. tropicalis	0.25		0.5			1			
	A. flavus[a]								0.5	1
	A. fumigatus[a]								0.5	1
	A. niger[a]								0.25	0.5
	A. terreus[a]								0.12	0.25
MICA	*C. albicans*	**0.25**	**0.016**	0.5			**1**	**0.03**		
	C. dubliniensis								0.12	0.25
	C. glabrata	0.06	0.03	0.12			**0.25**	**0.06**		
	C. guilliermondii	2		4			8			
	C. krusei	0.25		0.5			1			
	C. lusitaniae								0.5	1
	C. parapsilosis	**2**	**0.002**	**4**	**0.004–2**		8	4		
	C. tropicalis	0.25		0.5			1			
VORI	*C. albicans*	0.12	0.12	0.25–0.5			**1**	**0.25**		
	C. glabrata								0.25	0.5
	C. krusei	0.5		1			2			
	C. parapsilosis	0.12	0.12	0.25–0.5			**1**	**0.25**		
	C. tropicalis	0.12	0.12	0.25–0.5			**1**	**0.25**		
	A. flavus								2	4
	A. fumigatus		1		2			4	1	2
	A. niger								2	4
	A. terreus								2	4
FLUZ	*C. albicans*	2	2		4	4	8	8		
	C. dubliniensis								0.5	1
	C. glabrata		0.002		**0.004–32**	**≤32**	64	64		
	C. guilliermondii								8	16
	C. lusitaniae								1	2
	C. parapsilosis	2	2		4	4	8	8		
	C. tropicalis	2	2		4	4	8	8		
POSA	*C. albicans*		0.06					0.12	0.06	0.12
	C. glabrata								1	2
	C. guilliermondii								0.5	1
	C. krusei								0.5	1
	C. lusitaniae								0.06	0.12
	C. parapsilosis		0.06					0.12	0.25	0.5
	C. tropicalis		0.06					0.12	0.12	0.25
	A. flavus								0.5	1
	A. fumigatus		0.12		0.25			0.5		
	A. niger								2	4
	A. terreus		0.12		0.25			0.5	1	2
ITRA	*C. albicans*		0.06					0.12		
	C. glabrata								4	8
	C. krusei								1	2
	C. lusitaniae								1	2
	C. parapsilosis		0.12					0.25		
	C. tropicalis		0.12					0.25	0.5	1
	A. flavus		1		2			4	1	2
	A. fumigatus		1		2			4	1	2
	A. nidulans		1		2			4		
	A. niger								4	8
	A. terreus		1		2			4	2	4

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