

Antifungal drug susceptibility and resistance

Low rate of cryptic species and antifungal resistance in *Candida* isolates causing candidaemia in MadridL.J. Marcos-Zambrano¹, P. Escribano¹, P. Muñoz¹, E. Bouza¹, J. Guinea Ortega¹¹Clinical Microbiology and Infectious Diseases, Gregorio Marañón Hospital, Madrid, Spain

Objectives: We used molecular techniques to identify and investigate the antifungal susceptibility of a large number of *Candida* spp isolates causing candidemia in patients admitted to our hospital during a 5.5-year period.

Methods: We studied 562 isolates causing candidemia in 500 patients admitted to Gregorio Marañón Hospital between January 2007 and July 2013. Strains were identified after amplification and sequencing of the ITS1-5.8S-ITS2 region and further tested for *in vitro* susceptibility to amphotericin B (AmB), fluconazole (FLU), posaconazole (POS), voriconazole (VOR), micafungin (MYC), and anidulafungin (AND) according to the EUCAST EDef 7.2 procedure. The EUCAST species-specific breakpoints were used to classify isolates as resistant. For species-drug combinations without breakpoints, the ECOFFs used as tentative breakpoints were as follows: VOR (*C. glabrata* and *C. krusei* >1 µg/ml), POS (*C. glabrata*, >1 µg/ml; *C. krusei* >0.5 µg/ml), MYC (*C. tropicalis* >0.06 µg/ml; *C. krusei* >0.25 µg/ml). We obtained the sequence of the HS1 and HS2 regions of *fks1* and *fks2* (*C. glabrata*) in isolates resistant to one or more echinocandins.

Results: Most cases were caused by *C. albicans* (47.3%) and *C. parapsilosis* (29%) (Table). Cryptic species of *C. guilliermondii* (*C. guilliermondii sensu stricto*, n=7, *P. caribbica*, n=1; *Kodamaea ohmeri*, n=1) and *C. parapsilosis* (*C. parapsilosis sensu stricto*, n=158; *C. orthopsilosis*, n=3; *C. metapsilosis*, n=1) complexes, which were only detected using molecular techniques, accounted for 1% of isolates. *Candida* spp. included isolates of *C. dubliniensis* (n=6), *C. kefyr* (n=2), *C. lusitanae* (n=2), and *P. anomala* (n=1). The antifungal susceptibility of the isolates is shown in the Table. None of the isolates was resistant to AmB (MIC >1 µg/mL). The overall rate of FLU resistance was 4%, ranging from 0% (2009) to 6.3% (2010). The rate of resistance to echinocandins (1.3%) was also low. The HS1 and HS2 regions of *fks1* in strains showing resistance to one or more echinocandins were sequenced (*C. albicans*, n=4; *C. krusei*, n=1; *C. tropicalis*, n=2). The only point mutation found was at position R647G in the HS1 region of *fks1* in a *C. tropicalis* strain.

Conclusions: The percentage of cryptic species of *Candida* found in a large collection of *Candida* isolates causing candidemia was low. Less than 5% of the isolates were FLU-resistant, and this rate was particularly low for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. The rate of resistance to echinocandins was also low (<1.5%).

Species	MIC ₉₀ , in µg/mL (% of resistance)					
	AmB	FLU	VOR	POS	MYC	AND
<i>C. albicans</i> (n=266)	1	0.25 (0.8%)	0.015 (0.8%)	0.03 (0.8%)	0.015 (1.5%)	0.015 (0.4%)
<i>C. parapsilosis</i> complex (n=162)	1	0.5 (0%)	0.015 (0%)	0.062 (0%)	2 (0%)	2 (0%)
<i>C. glabrata</i> (n=58)	1	64 (8.6%)	1 (5.1%)	1 (6.8%)	0.015 (0%)	0.031 (0%)
<i>C. tropicalis</i> (n=46)	1	0.5 (0%)	0.031 (0%)	0.031 (0%)	0.031 (4.3%)	0.031 (2.1%)
<i>C. krusei</i> (n=10)	1	64 (100%)	0.5 (0%)	0.25 (0%)	0.125 (10%)	0.062 (10%)
<i>Candida</i> spp. (n=20)	2	16 (15%)	0.5	0.25	1	2