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Epidemiology and Antifungal Resistance in Invasive Candidiasis

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Abstract
The epidemiology of *Candida* infections has changed over the last two decades: The number of patients suffering from such infections has increased dramatically and the *Candida* species involved have become more numerous as *Candida albicans* is replaced as an infecting agent by various non-*C. albicans* species (NAC). At the same time, additional antifungal agents have become available. The different Candida species may vary in their susceptibility for these various antifungals. This draws more attention to *in vitro* susceptibility testing. Unfortunately, several different test methods exist that may deliver different results. Moreover, clinical breakpoints (CBP) that classify test results into susceptible, intermediate and resistant are controversial between CLSI and EUCAST. Therefore, clinicians should be aware that interpretations may vary with the test system being followed by the microbiological laboratory. Thus, knowledge of actual MIC values and pharmacokinetic properties of individual antifungal agents is important in delivering appropriate therapy to patients

Introduction

In 2001, McNeil et al. reported that (in the USA) "from 1980 through 1997, the annual number of deaths in which an invasive mycosis was listed on the death certificate (multiple-cause mortality) increased from 1557 to 6534" (Table 1, Fig. 1) [1]. Augmentation of fungal infections had been published earlier i.e. by Beck-Sague and Jarvis [2] and Edmond et al. [3] for the USA and by Lamagni et al. [4] for England and Wales (Fig. 2). There are several possible reasons for this change. An important one might be the increase in lifespan in the populations of the developed world and the age related loss of immune-competence. An increase of systemic fungal infections is probably also due to more intensive treatment schemes for hematological and oncological patients causing prolonged neutropenic phases. Finally, more effective antibacterial treatments allow patients with infections to survive longer without necessarily overcoming the underlying diseases and thus leaving them susceptible to other opportunistic infections. Eggimann et al. [5] summarized prior surgery, acute renal failure, previous yeast colonization, neutropenia, antibacterial therapy, parenteral nutrition, and central venous catheters as risk factors for invasive Candidia infections.

Change in Epidemiology

The increase in incidence of *Candida* infections barely preceded the introduction of fluconazole in 1990. This azole agent combined good activity against *Candida* albicans with reduced toxicity as compared to i.e. polyene anti-fungals. It is orally and parentally available and has a reliable that means linear pharmacokinetic profile [6], which makes it easy to handle. Not surprisingly, fluconazole became the agent of choice for many fungal infections as well as for prophylactic purposes, at that time often being applied in rather low doses. Although there is inconclusive evidence, many experts in the field believe that it was the selective pressure exerted by this therapeutic concept that caused changes in the epidemiology [7]. While in earlier years, *C. albicans* was responsible most of the invasive fungal infections (Table 2) [8], gradually more and more non-*C. albicans* species (NAC) were found as offending agents (Table 3) [9-16]. While the data show similar tendencies in the prevalence of various *Candida* ssp. worldwide, considerable differences can be observed as well. However, these do not lend themselves to further interpretation since significant differences in the demographics of the patients observed seem to be obvious. This change in prevalence of various *Candida* spp. is nevertheless of clinical importance, since individual species vary in their susceptibility to various antifungal agents. While national and international surveillance is important to recognize trends in epidemiology it is, however, of utmost importance to gain knowledge about the local epidemiology as this information should guide the empiric therapy of patients.

Susceptibility Testing

Some of the already cited and many other studies have also reported on the in-vitro susceptibility of *Candida* spp. For various reasons it is difficult to assess the various results. There are currently several test methods for performing these assays. Standardized methods have been published by the Clinical and Laboratory Standards Institute (CLSI) of the USA [17, 18] and by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19]. The two broth dilution methods are not identical as methodological differences include glucose concentration, inoculum size, shape of microtitration wells (flat or round), and end-point reading (visual or spectrophotometric). However, it appears

Table 1. Ranking of underlying causes of deaths due to infectious diseases in the United States in 1980 and 1997 [1].

Rank	1980		1997	
	Type of infection	No. of deaths	Type of infection	No. of deaths
1	Respiratory tract	56,966	Respiratory tract	87,181
2	Septicemia	9,438	Septicemia	22,396
3	Kidney/UTI	8,006	HIV/AIDS	16,524
4	Heart	2,486	Kidney/UTI	13,413
5	Tuberculosis	2,333	Heart	5,577
6	Bacterial meningitis	1,402	Hepatobiliary	4,596
7	Gastrointestinal	1,377	Mycoses	2,370
8	Hepatobiliary	1,277	Tuberculosis	1,259
9	Perinatal	1,035	Gastrointestinal	1,053
10	Mycoses	828	Perinatal	820

Note: Categories of infectious diseases identified by anatomic site rather than by causative microorganism did not have any microorganism specified in the death-certificate data. UTI = urinary tract infedtion.

Table 2. Species distribution of Candida from cases of invasive candidiasis[a] [8].

Species	% of total cases[b]					
	1997-1998	1999	2000	2001	2002	2003
C. albicans	73.3	69.8	68.1	65.4	61.4	62.3
C. glabrata	11.0	9.7	9.5	11.1	10.7	12.0
C. tropicalis	4.6	5.3	7.2	7.5	7.4	7.5
C. parapsilosis	4.2	4.9	5.6	6.9	6.6	7.3
C. krusei	1.7	2.2	3.2	2.5	2.6	2.7
C. guilliermondii	0.5	0.8	0.8	0.7	1.0	0.8
C. lusitaniae	0.5	0.5	0.5	0.6	0.5	0.6
C. kefyr	0.2	0.4	0.5	0.4	0.4	0.5
C. rugosa	0.03	0.03	0.2	0.7	0.6	0.4
C. famata	0.08	0.2	0.5	0.2	0.4	0.3
C. inconspicua			0.08	0.1	0.2	0.3
C. novegensis			0.08	0.1	0.07	0.1
C. dubliniensis			0.01	0.08	0.1	0.05
C. lipolytica			0.06	0.06	0.06	0.08
C. zeylanoides			0.03	0.08	0.02	0.04
C. pelliculosa				0.06	0.05	0.04
Canida spp.	3.9	6.0	3.7	3.3	7.9	4.9
Total no. of cases	22,533	20,998	11,698	21,804	24,680	33,002

[a] Data compiled from the ARTEMIS DISK Surveillance Program, 1997 to 2003 (221).
[b] Includes all specimen types and all hospitals from a total of 127 different institutions in 39 countries.
[c] Candida species not otherwise identified.

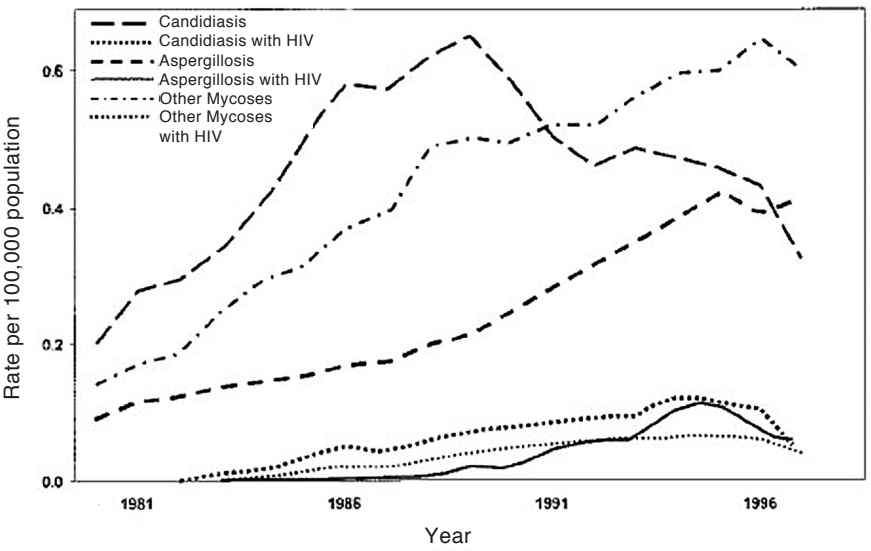

Fig. 1. Mortality in the United States, 1980-1997, due to candidiasis, and other mycoses in persons infected and persons not infected with HIV [1].

Table 3. Species distribution of Candida in blood stream infections in various studies.

Reference	9 Artemis	10 Sentry	11 Horn	12 Ostrosky	13 Cisterna	14 Arendrup	15 Fleck	16 Borg
Year	2005-2007	2008-2009	2004-2008	1995/1999	2008-2009	2004-2009	2004-2006	2004-2005
Location	worldwide	worldwide	USA	USA	Spain	Denmark	Germany	Germany
n	88647	1354	2019	2000	984	2901	512	561
C. albicans	65	48.4	45.6	36.7	49.1	57.1	43	58.5
C. glabrata	11.7	18.2	26	22.9	13.6	21.1	31.3	19.1
C. tropicalis	8	10.6	8.1	15.4	10.8	4.8	11.7	7.5
C. parapsilosis	5.6	17.1	15.6	19.6	20.7	3.7	5.7	8
C. krusei	2.5	2	2.5	2.5	2.1	4.1	3.7	1.4
C. guilliermondii	0.6		0.3					1.1
C. lusitaniae	0.6		0.8	1			≤1	0.2
C. kefyr	0.6						≤1	
C. inconspicua	0.3						≤1	1.1
C. famata	0.3						≤1	0.7
C. rugosa	0.2							0.2
C. dubliniensis	0.2		0.4	0.9		2.6		1.1
C. norvegensis	0.1							0.4
C. lipolytica	0.06						≤1	
C. sake	0.08						≤1	
C. pelliculosa	0.05							
C. apicola	0.06							
C. zeylanoides	0.02							
C. valida	0.01						≤1	
C. intermedia	0.01						≤1	0.4
C. pulcherrima	<0.01							
C. haemulonii	<0.01							
C. stellatoidea	<0.01							
C. utilis	<0.01						≤1	0.4
C. humicola	<0.01							
C. lambica	<0.01							
C. ciferrii	<0.01							
C. colliculosa	<0.01						≤1	0.4
C. holmii	<0.01							
C. marina	<0.01							
C. sphaerica	<0.01							
Candida spp.	4		0.7		3.6	5.1	4.7	

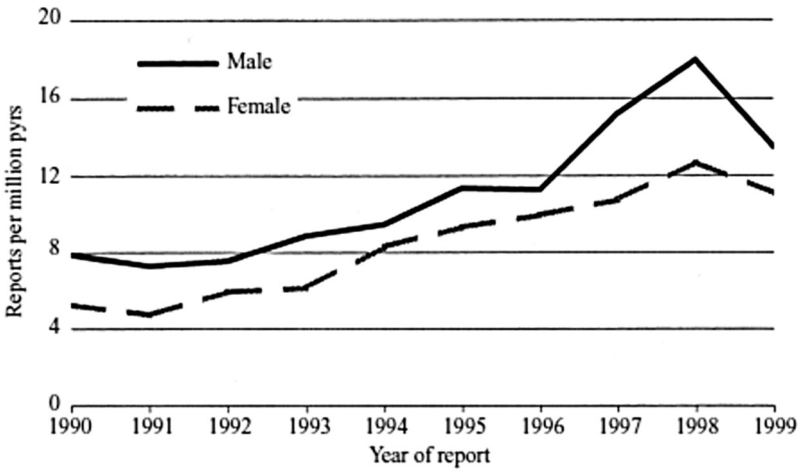

Fig. 2. Annual rates of candidosis laboratory reports, by sex (England and Wales: 1990-9) [4].

that they result in similar MIC levels for polyenes, azoles and echinocandins for identical isolates with a few noted exceptions [20-23]. This is especially true if isolates with defined resistance mechanisms are being tested [22, 23]. However, some "drug/bug" combinations seem to offer particular test problems i.e. Caspofungin and *C. glabrata* [22]. Moreover, EUCAST (and for that matter Etest) results have a tendency for one to two dilution steps lower MIC values [20, 23]. There are also some commercially available test devices that have been tested in their performance. A high degree of correlation with the reference methods was found for the Etest by various authors [22, 23]. The percentage of strains classified as resistant *in vitro* by the EUCAST procedure and as susceptible *in vitro* by the VITEK 2 system was 2.6%, and as resistant by the CLSI method and as susceptible by the VITEK 2 was 1.6% (very major error) [24]. The difference observed for the Vitek 2

Table 4. In vitro susceptibilities of Candida spp. to fluconazole and voriconazole as determined by CLSI disk diffusion testing[a] [9].

Species	Fluconazole[b]			Voriconazole[b]		
	No. of isolates	% S	% R	No. of isolates	% S	% R
C. albicans	128,625	98.0	1.4	125,965	98,5	1.2
C. glabrata	23,305	68.7	15.7	22,968	82.9	10.0
C. tropicalis	15,546	91.0	4.1	15,198	89.5	5.4
C. parapsilosis	12,788	93.2	3.6	12,453	97.0	1.8
C. krusei	5,079	8.6	78.3	5,oo5	83.2	7.6
C. guilliermondii	1,410	73.5	11.4	1,375	90.5	5.7
C. lusitaniae	1,233	92.1	5.4	1,215	96.7	2.0
C. kefyr	1,044	96.5	2.7	1,032	98.7	0.9
C. inconspicua	566	22.6	53.2	563	90.6	3.9
C. famata	622	79.1	10.3	606	90.3	5.0
C. rugosa	603	49.9	41.8	580	69.3	21.2
C. dubliniensis	310	96.1	2.6	308	98.4	1.0
C. norvegensis	248	41.9	40.7	247	91.5	4.0
C. lipolytica	130	66.2	28.5	128	77.3	14.1
C. sake	87	85.1	11.5	87	92.0	6.9
C. pelliculosa	87	89.7	6.9	86	94.2	4.7
C. apicola	57	98.2	1.8	57	98.2	1.8
C. zeylanoides	70	67.1	24.3	67	85.1	6.0
C. valida	21	23.8	61.9	22	81.8	13.6
C. intermedia	24	95.8	4.2	25	100.0	0.0
C. pulcherrima	14	100.0	0.0	14	100.0	0.0
C. haemulonii	9	88.9	11.1	9	88.9	11.1
C. stellatoidea	7	85.7	0.0	7	85.7	14.3
C. utilis	6	83.3	0.0	7	100.0	0.0
C. humicola	6	50.0	50.0	6	50.0	33.3
C. lambica	5	0.0	80.0	5	40.0	20
C. ciferrii	2	50.0	50.0	2	50.0	0.0
C. colliculosa	2	100.0	0.0	2	100.0	0.0
C. holmii	1	100.0	0.0	1	100.0	0.0
C. marina	1	0.0	0.0	1	100.0	0.0
C. sphaerica	1	100.0	0.0	1	100.0	0.0
Candida spp.	9,744	86.2	8.9	9,577	93.6	4.1

[a] Isolates were obtained from 133 istitutions, 2001 tp 2007.
[b] Fluconazole and voriconazole disk diffusion testing was performed in accordance with CLSI document M44-A (7). The interpretive breakpoint (zone diameters) were as follows: S, ≥19 mm (fluconazole) and ≥ 17 min (voriconazole); R, ≤14 mm (fluconazole) and ≤ 13 mm (voriconazole).
[c] Candida species, not otherwise specified.

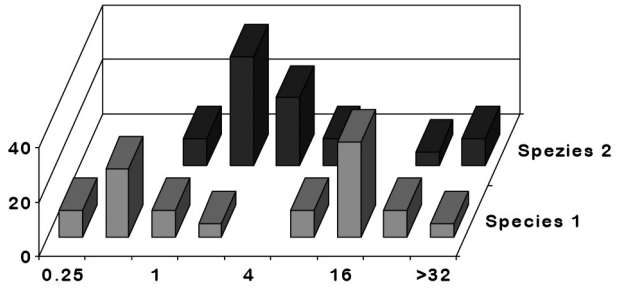

Fig. 3. Two very different fictitious MIC distributions resulting in the same MIC50/90 values: 2 and 32 mg/L

results and CLSI and EUCAST is driven by the fact that there are differences in clinical breakpoints (CBP) suggested by the two organizations. However these results indicate that although the CBP of EUCAST and CLSI are significantly different, currently only few strains are affected by this difference.

CBP are used to classify MIC results into susceptible (S), intermediate (I) (CLSI for some strange reason "susceptible dose dependent"; S-DD), and resistant (R), respectively. This has complicated to assess and compare studies were only percentages of S, I and R are published. The same is true for published MIC50/90 values, as very different MIC distributions might be behind these numbers. To illustrate this further, two very different fictitious distributions resulting in the same MIC50/90 values are given with Figure 3. Therefore, meaningful surveillance data should be published as MIC distributions. That allows for evaluation of the results even at a later date when i.e. CBP had been changed as has happened in the past. To circumvent the problem with differences in CBP published by various organizations, it has been suggested to use epidemiological cut -off values to distinguish between wild type (WT) organisms without any resistance mechanisms and non-wild type (N-WT) strains with higher MIC values that are thought or known to possess a resistance mechanism [22, 25]. This would put *in vitro* test results on the safe side as long as the particular species is a target for the antifungal agent in question. Obviously, CBP should not divide WT distributions as this will cause arbitrary test results. Monitoring the development of N-WT strains in surveillance studies allows one to analyze the spread of resistance mechanisms.

Table 5. In vitro susceptibilities of 5,346 clinical isolates of *Candida* spp. to anidulafungin, caspofingin, and micafungin [38].

Organism	No. of isloates tested	Antifungal agent	Cumulative % of isolates susceptible at a MIC (μg/ml) of[a]										
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	2,869	Anidulafungin	6.2	33.5	69.5	**92.4**	99.1	99.5	99.5	99.6	100.0		
		Caspofungin	1.7	26.7	74.2	**97.1**	99.3	99.9	100.0				
		Micafungin	11.9	80.6	**96.4**	99.3	99.4	99.5	99.6	100.0			
C. parapsilosis	759	Anidulafungin			0.3	0.3	0.3	1.4	4.7	27.9	**92.5**	100.0	
		Caspofungin		0.1	0.5	3.3	10.7	52.2	89.5	**98.6**	99.9	100.0	
		Micafungin		0.1	0.3	0.3	0.5	6.1	24.4	79.3	**100.0**		
C. glabrata	*747*	Anidulafungin		0.4	7.8	62.4	**93.6**	99.4	99.7	99.9	99.9	100.0	
		Caspofungin		7.0	65.2	**95.3**	98.4	99.2	99.7	99.9	99.9	99.9	100.0
		Micafungin	13.7	**91.4**	97.9	98.9	99.5	99.9	99.9	100.0			
C. tropicalis	*625*	Anidulafungin	3.2	24.2	75.7	**95.0**	98.4	99.4	99.5	99.5	100.0		
		Caspofungin	1.3	31.0	79.7	**97.3**	99.0	99.7	99.7	99.8	99.8	99.8	100.0
		Micafungin	4.0	39.5	77.6	**96.3**	98.6	99.5	99.7	100.0			
C. krusei	*136*	Anidulafungin		2.9	47.1	**90.4**	99.3	99.3	100.0				
		Caspofungin		0.7	0.7	41.9	75.7	**94.9**	99.3	100.0			
		Micafungin		2.2	13.2	85.3	**96.3**	100.0					
C. guilliermondii	61	Anidulafungin					3.3	6.6	13.1	57.4	**90.2**	100.0	
		Caspofungin			1.6	4.9	11.5	39.3	80.3	**95.1**	95.1	95.1	100.0
		Micafungin		3.3	3.3	6.6	11.5	21.3	65.6	**98.4**	100.0		
C. lusitaniae	58	Anidulafungin				1.7	13.8	43.1	**96.6**	100.0			
		Caspofungin			3.4	6.9	44.8	89.7	**96.6**	100.0			
		Micafungin			1.7	8.6	63.8	**96.6**	98.3	100.0			
C. kefyr	37	Anidulafungin		2.7	10.8	56.8	**100.0**						
		Caspofungin	13.5	**97.3**	100.0								
		Micafungin		5.4	40.5	**100.0**							
C. famata	24	Anidulafungin		4.2	16.7	20.8	20.8	20.8	25.0	50.0	**100.0**		
		Caspofungin		4.2	12.5	20.8	37.5	70.8	70.8	**95.8**	100.0		
		Micafungin		4.2	16.7	16.7	20.8	33.3	75.0	**91.7**	100.0		
Candida spp.	30	Anidulafungin	3.3	30.0	50.0	63.3	63.3	73.3	86.7	**93.3**	96.7	96.7	100.0
		Caspofungin		16.7	43.3	63.3	73.3	**96.7**	100.0				
		Micafungin		20.0	53.3	66.7	66.7	86.7	**100.0**				
Total	5,246	Anidulafungin	3.7	21.1	48.9	72.6	82.0	83.4	84.7	88.7	**98.8**	99.9	100.0
		Caspofungin	1.2	19.7	59.4	79.6	84.0	**91.9**	98.1	99.7	99.9	99.9	100.0
		Micafungin	8.8	60.9	75.6	81.3	83.3	85.0	88.5	**97.0**	100.0		

[a] Values corresponding to MICs at which at least 90% of isolates are inhibited are listed in bold types.

Table 6. In vitro antifungal agent susceptibilities of *Candida* and *Cryptococcus* isolates collected by the SENTRY Program in 2006 to 2007 [39].

Species (no. of isolates) and drug	MIC_{50}/MIC_{90} (μg/ml)	MIC range (μg/ml)	% by category[a]		
			S	SDD	R[b]
C. parapsilosis (238)					
Anidulafungin	2/2	0.03-4	95.4	–	4.6
Caspofungin	0.5/1	0.06-4	99.6	–	0.4
Amphotericin B	1/1	0.25-1	99.6	–	0.4
5-FC	≤0.5/≤0.5	≤0.5->64	98.7	(0.0)	1.3
Fluconazole	1/4	≤0.5-32	96.6	3.4	0.0
Itraconazole	0.25/0.25	≤0.015-2	40.8	57.1	2.1
Posaconazole	0.12/0.25	≤0.06-1	–	–	–
Voriconazole	≤0.06/0.12	≤0.06-2	99.6	0.4	0.0

Resistance Mechanisms

A number of different resistance mechanisms have been described in *Candida* spp. Often, several of these mechanisms are combined to result in a stepwise development of clinically relevant resistance. Resistance to i.e. fluconazole can be caused i.e. by alterations in sterol biosynthesis, by mutations in the drug target enzyme, sterol 14α-demethylase, which lowers its affinity for fluconazole, by increased expression of the ERG11 gene encoding for this enzyme, or by overexpression of genes coding for membrane transport proteins of the ABC transporter (*CDR1/CDR2*) or the major facilitator (*MDR1*) superfamilies [26].

Similarly *Candida* isolates were found with reduced susceptibility to echinochandins that showed mutations in selected regions of *fks1*, the gene encoding the echinocandin target enzyme 1-3-b-D-glucan synthase [27]. In particular, mutations of the serine at position 645 and also, in some cases, at position 641

Table 7. Antifungal susceptibilities of rare *Candida* bloodstream isolates [41].

Species	No. of isolates	Antifungal agent	No. inhibited at MIC (μg/ml) of:													
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16[b]	32	64[c]	≥128
C. lusitaniae	171	Amphotericin[a]				3	18	74	66	7	1	0	1	1			
	171	Fluconazole					20	52	64	20	5	3	1	1	3	2	
	171	Posaconazole	2	25	63	58	15	4	1	3							
	171	Voriconazole	123	33	3	4	2	3	2	1							
	96	Anidulafungin				5	13	36	40	2							
	166	Caspofungin		1	4	6	68	66	17	3	0	1					
	80	Micafungin	1	0	4	8	44	21	1	1							
C. guilliermondii	174	Amphotericin	1	0	1	8	63	62	24	6	2	1	1	0	0	5	
	175	Fluconazole						1	4	4	69	68	13	7	2	3	4
	175	Posaconazole		1	9	10	44	73	25	3	4	0	0	6			
	175	Voriconazole	2	11	19	83	43	8	1	0	2	0	0	6			
	107	Anidulafungin		1		2	6	5	7	37	41	8					
	156	Caspofungin			2	9	21	33	58	21	4	2	2	4			
	96	Micafungin		3	1	4	10	13	33	27	4	0	0	1			
C. orthopsilosis	102	Amphotericin						7	29	35	23	8					
	102	Fluconazole						6	28	45	9	8	4	1	0	1	
	102	Posaconazole		1	12	40	30	8	11								
	102	Voriconazole	1	24	40	24	1	10	1	1							
	52	Anidulafungin						3	12	28	9						
	91	Caspofungin	1	0	3	17	37	25	8								
	51	Micafungin					2	25	21	3							
C. kefyr	74	Amphotericin							20	43	10	0	0	0	0	1	
	74	Fluconazole					11	44	12	6	1						
	74	Posaconazole	1	3	17	21	23	8	1								
	74	Voriconazole	50	18	4	2											
	58	Anidulafungin		1	5	31	21										
	74	Caspofungin	11	56	6	1											
	53	Micafungin		4	22	26	1										
C. pelliculosa	40	Amphotericin					3	14	21	2							
	40	Fluconazole								2	7	24	7				
	40	Posaconazole				1	1	6	4	14	12	2					
	40	Voriconazole		1	1	1	21	13	3								
	14	Anidulafungin	2	9	2	1											
	37	Caspofungin	1	16	17	3											
	14	Micafungin		5	7	2											
C. famata	16	Amphotericin					1	6	8	0	0	1					
	16	Fluconazole								1	5	6	1	3			
	16	Posaconazole		2	0	0	1	5	7	1	2						
	16	Voriconazole			2	5	4	3	0	1	1						
	16	Anidulafungin				2	0	0	0	5	9						
	16	Caspofungin			1	2	2	5	3	2	1						
	16	Micafungin			1	1	0	2	5	5	2						
C. metapsilosis	30	Amphotericin					1	5	12	9	2	1					
	30	Fluconazole						1	0	19	9	1					
	30	Posaconazole		1	7	15	5	1	0	1							
	30	Voriconazole	1	4	22	2	1										
	11	Anidulafungin						5	3	2	1						
	24	Caspofungin			1	5	14	3	0	1							
	11	Micafungin						7	3	1							
C. dubliniensis	18	Amphotericin				1	8	7	1	1							
	18	Fluconazole					8	9	0	0	0	0	1				
	18	Posaconazole		4	6	7	1										
	18	Voriconazole	11	5	2												
	11	Anidulafungin			7	2	0	2									
	17	Caspofungin		2	6	9											
	9	Micafungin		4	3	2											
C. lipolytica	16	Amphotericin							1	5	5	4	1				
	16	Fluconazole							1	1	6	6	1	0	0	1	

Continued on following page

have been associated with decreased susceptibility to echinochandins [28].

It should be noted that there is at least for fluconazole a clear relation between MIC values, pharmacokinetics (expressed in serum AUC) and outcome (Fig. 4) [29]. Since resistance development against i.e. echinocandines so far is very limited, it is obviously very difficult to establish such a correlation. This problem is further aggravated by the fact that current antifungals still leave much to be desired as they i.e. do not reach the cure rates of antibacterial agents for susceptible bacteria. Moreover, the described resistance mecha-

Table 7 continued

Species	No. of isolates	Antifungal agent	No. inhibited at MIC (μg/ml) of:													
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16[b]	32	64[c]	≥128
	16	Posaconazole					2	1	8	4	0	1					
	16	Voriconazole		1	5	7	2	0	0	1							
	10	Anidulafungin						3	4	2	1						
	15	Caspofungin					6	9									
	10	Micafungin						6	3	1							
C. rugosa	16	Amphotericin							4	3	7	1	0	0	0	1	
	16	Fluconazole							1	0	4	3	2	4	1	1	
	16	Posaconazole				6	2	5	3								
	16	Voriconazole	2	1	4	3	1	3	2								
	16	Anidulafungin				3	1	4	3	3	0	0	2				
	16	Caspofungin			1	1	0	1	2	8	0	1	0	2			
	16	Micafungin			1	3	3	5	2	0	0	0	0	2			

[a] Amphotericin B MICs were determined by Etest.
[b] For posaconazole, voriconazole, anidulafungin, caspofungin, and micafungin, isolates for which MICs are reported to be 16 μg/ml encompass all isolates for which MICs were >8 μg/ml.
[c] For amphotericin B, isolates for which the MIC is reported to be 64 μg/ml encompass all isolates for which MICs were >32 μg/ml.

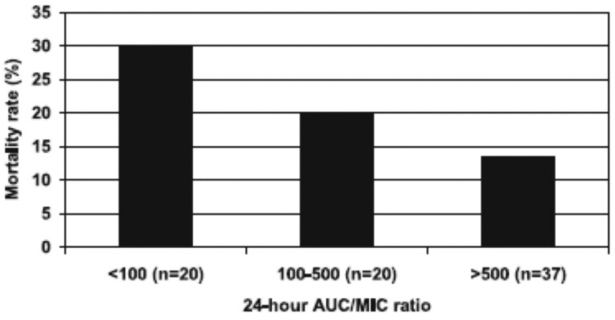

Fig. 4. Mortality rate stratified by tertiles and fluconazole AUC/MIC at 24 h (P = 0.09 using logistic regression controlling for time to initiation of fluconazole therapy) [29].

nisms were at least in individual cases associated with clinical failure of the involved patients [30-35]. This has raised concern over the CBP as suggested by the CLSI. For a discussion of this subject see [36, 37].

Resistance Surveillance

Among the worldwide largest and long run surveillance systems is the ARTEMIS program. In a recent publication [9], comparative susceptibility data for fluconazole and voriconazole for more than 190,000 isolates collected from 2001 to 2007 were provided and analysis of resistance rates by year, geographic location, hospital location, and specimen type for selected species were included. The data were collected employing the CLSI disk diffusion method and are summarized in Table 4. They show that fluconazole resistance has to be expected especially in *C. glabrata*, *krusei*, *guillermondii*, *famata*, *inconspicua*, *rugosa*, *norvegensis* and some other rarer species. There seem to be some but no complete cross-resistance with voriconazole which leaves the latter as an option in appropriate cases. A trend toward increased resistance over the most recent 3 years (2005 to 2007) was observed for voriconazole and some species with low prevalence such as *C. famata* (1.1% to 5.7%), *C. norvegensis* (0.0% to 6.9%), *C. lipolytica* (0.0% to 11.1%), and *C. pelliculosa* (14.3% to 16.7%). However, there was no trend toward increased resistance to voriconazole among the fluconazole-resistant species *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. rugosa*, and *C. inconspicua*.

MIC distributions for echinocandins have been published by Pfaller et al. (Table 5) [39]. The results of this study demonstrate the comparable spectrum and potency of all three available echinocandin antifungals against a large collection of clinically important *Candida* spp. It also highlights the fact that species such as *C. parapsilosis* and *C. guilliermondii* exhibit decreased susceptibilities to all three echinocandins. The clinical relevance of these elevated MICs currently remains doubtful.

The data collected by the SENTRY surveillance program were published as MIC50/90 values and interpreted results according to CLSI, only [39, 40]. However, this study included most important antifungals. An excerpt for *C. parapsilosis* is given with Table 6. German data were published in the same way [15], not offering significant differences to the SENTRY results with the exception of a high degree of flucytosine resistance in *C. krusei* and *C. tropicalis*. Finally, susceptibility data for rare *Candida* isolates have been collected by Diekema et al. [41] (Table 7) and Chen et al. [42] that might help to guide therapy in these cases.

Conclusion

Meanwhile *in vitro* methods are available to assess reliably the susceptibility of fungal isolates. There are, however, considerable differences in the evaluation of the results, as CLSI and EUCAST breakpoints vary. If isolates with known resistance mechanisms that have been shown to be clinically relevant at least in individual cases shall not be categorized as susceptible, some CLSI CBP need to be reconsidered. Despite the fact that a number of new antifungals are nowadays available, clinical results of antifungal therapy leave much to be desired. Hence, optimization of empiric therapy according to the local epidemiological situation and reevaluation of the therapeutic regimen when susceptibility results become available should carefully be followed. With our expanded knowledge on pharmacokinetics of antifungal compounds, MIC data could be valuable at least when treating invasive fungal infections. More information about MICs of clinical iso-

lates and outcome of the particular patients would be helpful to establish further and validate current CBP.

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