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Echinocandin resistance: an emerging clinical problem?

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Abstract

Purpose of review—Echinocandin resistance in *Candida* is a great concern, as the echinocandin drugs are recommended as 1st line therapy for patients with invasive candidiasis. Here we review recent advances in our understanding of the epidemiology, underlying mechanisms, methods for detection and clinical implications.

Recent findings—Echinocandin resistance has emerged over the recent years. It has been found in most clinically relevant *Candida* species, but is most common in *C. glabrata* with rates exceeding 10% at selected institutions. It is most commonly detected after 3–4 weeks of treatment and is associated with a dismal outcome. An extensive list of mutations in hot-spot regions of the genes encoding the target has been characterised and associated with species and drug specific loss of susceptibility. The updated antifungal susceptibility testing reference methods identify echinocandin resistant isolates reliably; while the performance of commercial tests is somewhat more variable. Alternative technologies are being developed including molecular detection and MALDI-TOF.

Summary—Echinocandin resistance is an increasingly encountered and mandates susceptibility testing particularly in patients with prior exposure. The further development of rapid and user-friendly commercially available susceptibility platforms is warranted. Antifungal stewardship is important in order to minimise unnecessary selection pressure.

Keywords

echinocandins; *Candida*; *FKS* hot spot mutation; epidemiology

Introduction

The three echinocandins, anidulafungin, caspofungin and micafungin, have been available for a decade. They display *in vitro* fungicidal activity against most *Candida* species, attractive tolerability and pharmacokinetic profiles, and have been recommended as first line agents for invasive candidiasis (1–6). The echinocandins exhibit their antifungal activity via inhibition of the enzyme glucan synthase encoded by three related genes (*FKS1*, *FKS2* and *FKS3*). Most *Candida* species are considered good targets. However, some *Candida* spp. are

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inherently less susceptible *in vitro* due to naturally occurring polymorphisms in the target protein (Table 1) (7). For example, the echinocandin MIC is approx. 7-fold higher against *C. parapsilosis*, which has a naturally occurring alteration in the target gene (Table 1). Following regulatory approval, the use of the echinocandins for prophylaxis and treatment has grown substantially. A consequence of enhanced drug exposure is increased selection pressure for resistance and indeed an altered species distribution for invasive infections has been linked to the echinocandin use (8). Moreover, reports on acquired echinocandin resistance, defined as resistance among species that are normally susceptible, have emerged and today acquired resistance has been reported in single isolates belonging to most *Candida* species including *C. albicans*, *C. dubliniensis*, *C. kefyr*, *C. glabrata*, *C. krusei*, *C. lusitaniae* and *C. tropicalis* (9–15). Elevated MICs have been associated with a number of single amino acid (AA) substitutions caused by mutations in specific “hot spot” regions of the well conserved target genes *FKS1* for all *Candida* spp., as well as *FKS2* for *C. glabrata* (Table 1) (16). The position, as well as, the specific AA substitution determines the degree of MIC elevation in the individual isolate.

The aim of this review is to present an up to date overview of echinocandin resistance in *Candida* by addressing the current epidemiology, the underlying molecular mechanisms and their differential impact on susceptibility and fitness, methods for resistance detection and the potential implication of increasing echinocandin resistance on treatment strategies of invasive candidiasis.

Epidemiology

Quite soon after the introduction of the echinocandins it was noted that the increasing use was accompanied by epidemiological shifts with a proportional or numeric increase in the less susceptible *C. parapsilosis* at several centres (8;17;18). Subsequently, the number of reports of acquired echinocandin resistance has attracted attention. Most are case reports or case series, which collectively document the potential of resistance development in almost all species that are not intrinsically susceptible (9–15). Although some cases are reported after short-term use (a week)(13;19), most cases are diagnosed after 3–4 weeks of therapy or even later (12;20;21). Resistance is more often found in *C. glabrata*, although this species is less frequent than *C. albicans* as a cause of invasive infections and has been reported on both sides of the Atlantic ocean (Fig 1) (21;22). Whether this is due to a higher potential for developing resistance mutations or it relates to patients with *C. glabrata* infections who more often receive prolonged echinocandin therapy due to the intrinsic reduced susceptibility to azoles like fluconazole remains to be understood. Nevertheless, resistance in *C. glabrata* is on the rise. This was recently documented in a 10-year survey at the Duke University hospital where the echinocandin resistance rate increased from 4.9% to 12.3% in 2001–10 (23). A similar trend has been observed in the nation-wide fungaemia survey in Denmark, although on a smaller scale. Here, no cases were found in 2004–7, whilst 0.9% of the *C. glabrata* blood stream infections involved resistant isolates in 2008–9, 1.2% in 2010–11 and 3.1% resistance in 2012–13 (Arendrup, unpublished observations). Of note, resistance figures observed in fungaemia surveillance programmes may underestimate the true number of such cases, since only the initial blood isolate is included to avoid biasing the data set, which is sound from an epidemiological point of view. Notably, it was recently

reported that echinocandin resistance was detected in as many as 10% of post treatment oral mucosal isolates obtained from candidaemic patients initially treated with an echinocandin (24).

The use of echinocandins as 1st line agents for candidaemia has unquestionably improved outcome for patients infected with a susceptible isolate (1). However, there is solid documentation that the outcome for patients with increased MICs is significantly poorer. Not only has an unsuccessful outcome been reported in most case reports, but it was also documented in two recent studies that 80% and 78.5% candidaemia patients with an echinocandin resistant *C. glabrata* isolate fail therapy, respectively (23;25).

Mechanisms and impact of the MIC

Intrinsic target gene alterations are found in *C. parapsilosis*, *C. metapsilosis*, *C. orthopsilosis*, *C. guilliermondii* and *C. lipolytica* (Table 1) (7;26). Additionally, polymorphisms occur at codon I660 in hot spot 1 of *FKS1* in *C. krusei*, for which the *in vitro* micafungin MIC is elevated approx. 2-fold and at codon V641 in *FKS1* and V1374 in *FKS2* in *Saccharomyces cerevisiae*, which is in general slightly less echinocandin susceptible than *C. albicans* and *C. glabrata*. The potential impact of these polymorphisms for therapeutic response remains unclear (Table 1).

Acquired *FKS* alterations are most commonly substitutions, but deletions and stop codons have also been reported in *C. glabrata* (Table 1) (16;21). The degree of the MIC elevation depends on the position as well as the specific AA substitution. The most significant MIC elevation is found for alterations involving the 1st and 5th AA(F (phenylalanine) and S (serine)), respectively in the hot spot 1 regions of the *FKS1* or *FKS2* target genes (Table 1 and 2). *FKS* alterations in most cases confer cross-resistance to all three echinocandins. However, some alterations cause more moderate MIC elevations and not always for all three compounds (Table 2). For example, the F659-DEL in *C. glabrata* confers resistance to all three echinocandins, whereas the F659S substitution at the same codon gives rise to anidulafungin and caspofungin resistance, whereas the micafungin MIC and *in vivo* efficacy in a murine animal model remains unchanged (23;25;27–30). Finally, the MIC increase caused by a specific alteration may be species specific. Thus, the echinocandin MICs were elevated at least 5-foldabove the breakpoints for *C. krusei* harbouring the D662Y compared to 1-foldabove the breakpoint for *C. albicans* and an MIC below the breakpoint for *C. glabrata* harbouring the corresponding alterations (D648Y in *C. albicans* and D666Y in the Fks2p protein for *C. glabrata*)(20;28).

Implication on fitness

Resistance mutations often come at a fitness cost and when that is the case, it may limit the spread of the organism due to competition from wild type isolates when therapy is discontinued. Echinocandin resistance in *Candida* has been linked to loss of fitness for homozygous Fks1p F641 and S645 mutants of *C. albicans* and Fks2p S663P mutants of *C. glabrata* (31–33). Thus, mutations at these hot spots have been linked to a) impaired enzyme capacity, b) altered cell wall composition, i.e. reduced glucan and increased chit in content, c) increased cell wall thickness and d) impaired filamentation properties, which contribute to

a lower growth rate *in vitro* and *in vivo* in drosophila and mouse models (31–33). Similarly, Lackner et al found reduced kidney burden comparing sequential *C. albicans* clinical isolates with and without two heterogenous mutations simultaneously at position R647 and P649 (34). In contrast, Borghi et al found no significant difference in virulence or fitness when comparing sequential patient *C. glabrata* isolates with and without the S663P mutation in the *Galleria mellonella* larvae model (35). Either, this contradictory finding relates to the different virulence models used, i.e. larvae versus mouse models, or alternatively, it may be explained by differences in the fitness reduction in the two isolates investigated in these studies. Indeed, whole genome sequencing of a series of clinical *C. glabrata* isolates revealed that a subsequent compensatory mutation (CDC55 P155S) mitigated the fitness cost induced by the S663P mutation (32).

Detection of resistance

Detection of echinocandin resistance can be assessed phenotypically, using microbroth dilution, Etest or disk diffusion or semi-automated systems like the VITEK system or it can be done molecularly by detection of mutations in the hot spot regions of the *FKS1* and *FKS2 (C. glabrata only)* genes. Additionally, preliminary studies have exploited the adoption of MALDI-TOF for resistance detection. These options and comments regarding pros and cons will be discussed below.

Microdilution Reference testing and commercial phenotypic susceptibility tests

The two organisations EUCAST and CLSI both have established reproducible and reliable microbroth dilution susceptibility tests for *Candida* and echinocandins (36–38). Besides being the reference method commercial susceptibility tests are standardised against, these reference methods have proven reliable and useful in mycology reference laboratories for the detection of resistance in referred clinical isolates. Recently, EUCAST breakpoints were developed for anidulafungin and micafungin and the former CLSI breakpoints were revised providing species specific breakpoints for all three echinocandins and their value confirmed (28;39–42). Despite this, some laboratories have noted an unexpected high number of *C. glabrata* and *C. krusei* isolates being categorised as non-susceptible using the CLSI method for testing caspofungin susceptibility and by using the Etest and Sensititre Yeast One system, which are standardised against the CLSI method (29;43–45). Thus, almost 20% of *C. glabrata* isolates and more than a third of the *C. krusei* isolates were anidulafungin and micafungin susceptible but caspofungin non-susceptible (44;45). This may in part be associated with variability in the caspofungin used for *in vitro* testing (46). Consequently, anidulafungin and micafungin have been evaluated as markers for caspofungin susceptibility and found appropriate for this purpose and is recommended also by EUCAST (40;47;48). The Sensititre Yeast One system was recently evaluated in a multicentre study using consecutive samples received in each of eight laboratories. Overall a good inter laboratory agreement was achieved and the resistance rates reported for anidulafungin and micafungin was within the expected range (4.3 and 4.4% for anidulafungin and micafungin, respectively) (45).

Few studies have systematically evaluated the commercial susceptibility test systems for their ability to correctly discriminate between susceptible wild type isolates and those bearing *FKS* resistance mutations by including a sufficient number of mutant isolates. In one such study evaluating the performance the VITEK system for caspofungin susceptibility, a relatively high number (19.4%) of mutant isolate were mis-classified as susceptible (49). This finding coupled with the fact that the system could not discriminate intermediate from susceptible *C. glabrata* isolates because the caspofungin concentration used in the system does not encompass the breakpoint, renders it less useful in its current form despite being very user friendly. Hence, although commercial testing has become available for most antifungal compounds including the echinocandins, some challenges remain to be addressed. Therefore, any laboratory wishing to perform antifungal susceptibility testing using one of these systems must assure that the MIC values generated for wild type isolates match the wild type distributions used for the clinical breakpoint setting, before adopting the reference breakpoints (38).

Molecular approaches

So far, clinically relevant acquired echinocandin resistance in *Candida* has never been detected in absence of *FKS* hot spot mutations. Hence, an attractive approach is to apply molecular tools for echinocandin resistance detection. Target gene sequencing has become increasingly available and appropriate primers designed and published for the most common species (9–15). The drawback, however, is the associated cost and it is time consuming unless automated. Various PCR formats focussing specifically on the detection of mutations in *C. glabrata* have been developed (50;51). The obvious benefit of such assays is that they allow a rapid detection of resistance in the species where resistance most often occur. The challenges are that a correct identification to the species level is required for selection of appropriate methodology and that knowledge regarding the species and compound dependent differential impact on the susceptibility is required to separate mutations associated with therapeutic failure from those that may respond to one or several echinocandins. Finally, none of these methods is yet commercially available and therefore implementation requires molecular biology expertise and in house validation.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF)

Recently, the potential of using MALDI-TOF detection of proteome changes after a 15-h exposure of fungal cells to serial drug concentrations was described for the determination of antifungal susceptibility (52). It was followed by a simplified version, which facilitated discrimination of susceptible and resistant isolates of *C. albicans* after only 3 hours of incubation in the presence of two “breakpoint” level drug concentrations (0.03 and 32 mg/L) of caspofungin (53). Categorizations determined using MALDI-TOF MS-based AFST (ms-AFST) were consistent with the wild-type and mutant FKS1 genotypes and the AFST reference methodology. Although it remains to be investigated how this approach can be extended to include other species with different growth kinetics and species specific clinical breakpoints, the prospect of a “same-day” susceptibility screening test is indeed attractive.

Clinical implications

The increasing incidence of echinocandin resistant *Candida* isolates is a reason for concern, as it is leading to the emergence of multidrug resistant organisms that present limited, if any, treatment options. This is particularly true for the emergence of echinocandin resistance in *C. glabrata*, an organism that is commonly resistant to azole drugs and for which amphotericin B is therefore the only therapeutic alternative. Echinocandin resistance often develop in a progressive manner. Resistant isolates can be detected in the oral flora post candidaemia treatment at a rate of approx. 10% often in co-existence with susceptible isolates but is detected at a lower rate and apparently typically later (3–4 weeks) among blood isolates (12;21;24). Unlike the azoles, echinocandins are not used in the primary health care sector or for plant and material protection. Therefore, antifungal stewardship in the hospital is of utmost importance in order to minimise the selection pressure when possible. A step down approach has been recommended in the new ESCMID guidelines for the management of invasive candidiasis (2–5). Based upon the data from initial controlled trial, ESCMID recommended de-escalation after 10 days, as data was not available supporting any earlier time-point (54). Recently, early step-down after 5 days of echinocandin therapy was found efficacious in an open-label study in patients meeting the prespecified criteria (ability to tolerate oral therapy; afebrile for > 24 hours; hemodynamically stable; not neutropenic; and with a documented clearance of *Candida* from the bloodstream) (55). This may suggest that early de-escalation is a reasonable approach at least in this category of patients. It is well recognised, that the clinical situation may mandate longer treatment and that voriconazole or high dose fluconazole may not be attractive alternatives in severely ill patients with *C. glabrata* candidaemia. Careful monitoring for antifungal resistance in invasive and colonising samples should be performed when echinocandin therapy is prescribed for longer time periods. Moreover, strategies involving alternating therapy (echinocandin and amphotericin B) or periods with combination therapy might potentially be alternative options that deserve further investigation.

Conclusion

Echinocandin resistance is emerging in population based candidaemia surveys and particularly in severely ill patients receiving longer-term therapy. It is particularly challenging with *C. glabrata* where the alternative treatment options are limited. The outcome for patients with resistant isolates on echinocandin therapy is dismal. Therefore, early detection is mandatory. Antifungal susceptibility testing has been optimised over the recent years and should be available real time for centres managing high-risk patients. However, further work is needed in order to optimise susceptibility testing in the routine setting using commercial test formats. New modalities including molecular and MALDI-TOF detection are under development and appear promising for future implementation in clinical microbiology laboratories. Antifungal stewardship is an important tool reducing unnecessary use and the treatment duration whenever clinically indicated may help reducing the selection pressure and thus reverting the rise in resistance. Potentially, strategies involving alternating or combination therapy might deserve clinical evaluation for patients

requiring long term antifungal treatment with *Candida* coverage in order to avoid selection of resistance to this important drug class.

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Key points

1. Echinocandin resistance in *Candida* is emerging in most clinically relevant *Candida* species but is particularly common in *C. glabrata*
2. A wide range of hot spot mutations in the FKS target genes have been characterised and their impact on MIC and fitness described
3. Reference antifungal susceptibility testing can reliably identify resistant isolates
4. New methodologies include molecular techniques and MALDI-TOF are being developed for detection of resistant isolates
5. Antifungal stewardship including efforts to limit long term echinocandin exposure is recommended

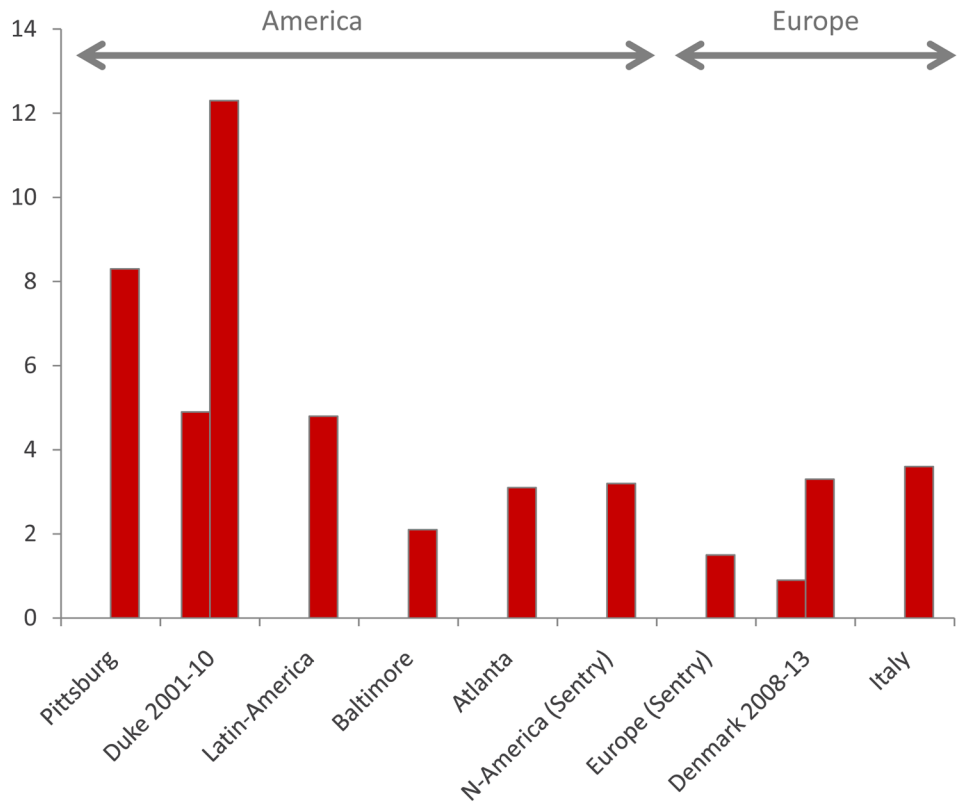

Fig. 1.

Echinocandin resistance in *C. glabrata* in Europe and America. Proportion of invasive isolate with resistance at tertiary centres (Pittsburgh, Duke and 7 Latin American hospitals), Shields AAC 2013; Alexander CID 2013; Nucci Plos One 2013; In population based surveys (Baltimore, Atlanta, Denmark) and Italy and from the global sentry study. Zimbeck AAC 2010; Pfaller JCM 2011; Arendrup Unpubl data; Tortorano Infection 2013.

Table 1

FKS amino acid (AA) sequences and ECOFFs for 12 wild type *Candida* species and *Saccharomyces cerevisiae*. AA alterations are underlined and in bold font with a colour indication of origin of the mutation and impact on the MIC (as explained in detail in the footnote).

	AFG EUCAST ECOFF (mg/L)	FKS1p				FKS2p			
		Hot spot 1		Hot spot 2		Hot spot 1		Hot spot 2	
		1st AA no.	AA sequence	1st AA no.	AA sequence	1st AA no.	AA sequence	1st AA no.	AA sequence
C. albicans	0.03	641	FLTLSLRDP	1357	DWIRRYTL				
C. dubliniensis	0.03	641	FLTLSLRDP	1357	DWIRRYTL				
C. glabrata	0.06	625	FLILSLRDP	1340	DWVRRYTL	659	LILSLRDP	1374	DWIRYTL
C. kefyr	(0.03)	54*	LTLSLRDP	769*	DWVRRYTL				
C. krusei	0.06#	655	FLILSIRDP	1364	DWIRRYTL				
C. lusitaniae	(0.06)	634*	FLTLSLRDP	**	DWIRRYTL				
C. tropicalis	0.06	76*	FLTLSLRDP	792*	DWIRRYTL				
C. parapsilosis	4	652	FLTLSLRDA	1369	DWIRRYTL				
C. metapsilosis	(4)	104*	FLTLSLRDA	821*	DWIRRYTL				
C. orthopsilosis	(4)	39*	FLTLSLRDA	756*	DWVRRYTL				
C. guilliermondii	(4)	632	FMALSLRDP	1347	DWIRRYTL				
C. lipolytica	NA	662	FLILSLRDP	1387	DWIRRCVL				
S. cerevisiae	(1)	639	FLVLSLRDP	1353	DWVRRYTL	658	FLILSLRDP	1372	DWVRRYTL

X “strong R” mutation, low letters indicate the codon involves a mutation or deletion; high letters indicate the codon involves a mutation or stop codon;

X “weak R” mutation;

X “silent” mutation, acquired or naturally occurring;

X naturally occurring mutation proven or possibly related to the intrinsic lower susceptibility;

X naturally occurring mutation of unknown impact;

*Inaccurate annotation, sequencing of entire gene-sequence required;

#Micafungin ECOFF elevated for *C. krusei* compared to *C. albicans* and *C. glabrata*, but not the anidulafungin ECOFF.

Table 2

Anidulafungin, caspofungin and micafungin MIC elevation for specific hot-spot 1 Fks2p alterations in *C. glabrata*.

FKS alteration	MIC (mg/L)			Dilution steps above the BP*			Method, Reference
	Anidulafungin	Caspofungin	Micafungin	Anidulafungin	Caspofungin	Micafungin	
F659							
F659-DEL	0.12–0.5	0.5–1[a]	0.06–0.5	1–3	2–3[a]	2–4	EUCAST/Etest[a], Arendrup unpublished data
	ND	8	ND	-	6	-	CLSI, Shields AAC 2013
	1	>8	2	4	>6	5	CLSI, Alexander CID 2013
F659V	1	2–8	0.12–0.25	3	4–6	1–2	CLSI, Castanheira AAC 2014
	1	2–4	0.25	3	4–5	2	CLSI, Alexander CID 2013
F659Y	1	1–2	0.12–0.25	3	2–4	1–2	CLSI, Castanheira AAC 2014
	0.5–1	0.5–2	0.25	2–3	2–4	2	CLSI, Pham AAC 2014
F659L	ND	1–2	ND	-	3–4	-	CLSI, Shields AAC 2013
F659S	0.12–0.5	0.5–2[a]	0.03	1–3	2–4[a]	0	EUCAST/Etest[a], Arendrup unpublished data
	0.25–1	0.25–0.5	0.06	1–3	1–2	0	CLSI, Castanheira AAC 2014
	2	2	0.25	4	4	2	CLSI, Pham AAC 2014
L662W	1–2	0.5–1	0.25–0.5	3–4	2–4	2–4	CLSI, Castanheira AAC 2014
S663							
S663P	0.25–2	>32[a]	0.12–1	2–5	>8	2–4	EUCAST/Etest[a], Arendrup unpublished data
	1–4	1–16	0.25–4	3–5	3–7	2–6	CLSI, Castanheira AAC 2014
	ND	0.5–8[b]	ND	-	2–6	-	Yeast one, Beyda CID 2014
	0.5–4	1–>16	0.5–4	2–5	2–>7	3–6	CLSI, Pham AAC 2014
	1–4	0.5–>8	0.12–>8	3–5	2–>6	1–>7	CLSI, Alexander CID 2013
S663Y	1	0.5	0.5	3	2	3	CLSI, Castanheira AAC 2014
S663F	0.12–1	0.5[a]	ND	1–4	2	-	EUCAST/Etest[a], Arendrup unpublished data
	0.5	0.25	0.12	2	1	1	CLSI, Castanheira AAC 2014

FKS alteration	MIC (mg/L)			Dilution steps above the BP*			Method, Reference
	Anidulafungin	Caspofungin	Micafungin	Anidulafungin	Caspofungin	Micafungin	
	ND	0.25	ND	-	1	-	Yeast one, Beyda CID 2014
	0.5–2	0.25–2	0.12–0.25	2–4	1–4	1–2	CLSI, Pham AAC 2014
L664R	1	0.25–0.5	0.25	3	1–2	2	CLSI, Castanheira AAC 2014
R665S	0.12	0.5	0.12	0	2	1	CLSI, Alexander CID 2013
R665G	0.25	0.5	0.12	1	2	1	CLSI, Alexander CID 2013
D666Y	0.12	0.25	0.06	0	1	0	CLSI, Castanheira AAC 2014
D666G	1	2	0.12	4	4	2	EUCAST, Arendrup unpublished data
D666E	0.25	2	0.06	2	4	1	EUCAST, Arendrup unpublished data
P667							
P667T	0.25	ND	0.03	2	-	0	EUCAST/Etest[a], Arendrup unpublished data
	1	1	0.12	3	3	1	CLSI, Alexander CID 2013
P667H	2	2	0.25	4	4	2	CLSI, Pham AAC 2014

*the echinocandin clinical breakpoints BPs applied are those for the specific reference method used and are as follows: EUCAST MIC testing: anidulafungin S: ≤ 0.06 mg/L, micafungin S: ≤ 0.03 mg/L and CLSI MIC testing: anidulafungin: S: ≤ 0.125 mg/L, caspofungin: S: ≤ 0.125 mg/L and micafungin S: ≤ 0.06 mg/L.

[a]EUCAST BPs have not yet been established for caspofungin, hence caspofungin susceptibility testing was done using Etest and applying the CLSI breakpoints as recommended by the manufacturer.