The role of aneuploidy in the emergence of echinocandin resistance in human fungal pathogen Candida albicans

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Fungal diseases largely affect human and animal health and dramatically diminish food crop yields [1]. Among fungi, systemic Candida infections are the second or third most common pathogens isolated from blood cultures in the USA [2]. Candida albicans is still the predominant Candida species, causing up to 50% of candidemia despite an increase in diversity of Candida species isolated from clinical samples [3]. In healthy individuals, C. albicans is a harmless inhabitant of mucosal surfaces throughout the body. However, in immune-compromised individuals, C. albicans can become a dangerous pathogen, causing severe or even fatal infections. In this review, we summarize recent data linking the reduced susceptibility of C. albicans cells to mainline echinocandin (ECN) drugs to aneuploidies of chromosomes 5 (Ch5) and Ch2.

Evolution of ECN resistance and mechanisms influencing susceptibility

The ECN drugs caspofungin, anidulafungin, and micafungin that are recommended as frontline therapy for candidiasis have few adverse actions and drug–drug interactions [4]. ECN drugs kill C. albicans cells by inhibiting glucan synthase, thus interfering with biosynthesis of the cell wall. Unlike well-studied multiple resistance mechanisms to fluconazole, another common antifungal from theazole class, there is only one generally recognized mechanism of clinical resistance to drugs from the ECN class. This mechanism involves point mutations in the essential FKS1 gene (orf19.2929) encoding a catalytic subunit of the 1,3-β-glucan synthase complex. Mutations are clustered in two “hotspot” regions, HS1 and HS2, encompassing residues from 641 to 649, and from 1,345 to 1,365, respectively [5]. Mutations in these regions cause dramatic elevation of C. albicans minimum inhibitory concentration (MIC) values to ECNs and reduce the sensitivity of the glucan synthase to up to 3,000-fold, the concentration of caspofungin inhibiting 50% of enzymatic activity (IC50) [6].

However, it has now become obvious that C. albicans possesses mechanisms independent of FKS1 mutations that can decrease susceptibility to ECNs, although these “alternative” mechanisms do not confer clinical resistance. These “alternative” mechanisms have been brought to light by dozens of clinical isolates of Candida species that display a wide range of increased MIC values for ECNs, including some at or below the MIC breakpoints, but, importantly, without canonical FKS1 mutations [7–10]. Consistent with these observations, several laboratories found that mutants lacking FKS1 mutations, but displaying (albeit relatively modest) 2 to 8 fold increases of MIC, can be easily generated in vitro on agar plates supplemented with caspofungin. While FKS1 mutations leading to resistance can also arise in vitro, these are typically rare [11–13]. Furthermore, while Cowen and colleagues observed evolution of ECN...
resistance in a series of isogenic clinical isolates of the related species Candida glabrata, they demonstrated that mutation to resistance was preceded by a mutation in a different gene that conferred a relatively small increase in MIC [14]. Indeed, quickly arising mutations conferring decreased susceptibility to ECNs are currently viewed as a means to provide a window of opportunity for temporary survival and subsequent formation of resistant FKS1 mutations. Based on such observations, Healey and Perlin [15] proposed a multistep model in which spontaneously acquired mutations lead to some decrease of drug susceptibility prior to acquisition of FKS1 resistance mutations and thus play an important role in the evolution of ECN resistance (Fig 1).

Subsequently, given their importance, some of the genes that confer decreased susceptibility have been identified by screening C. albicans deletion libraries against the ECN caspofungin [16]. In addition, such genes were also identified in mutants that were caspofungin-generated in vitro [17]. Clearly, understanding mechanisms that promote ECN resistance by influencing ECN susceptibility is of high importance. Although currently the incidence of clinical resistance to ECN drugs is relatively low, it is persistent, and the number of resistant cases is growing, primarily due to the increased use of ECNs for disease and prophylactic treatment.

Spontaneous aneuploidies of C. albicans control vital functions

C. albicans has a diploid genome, which is organized into eight pairs of chromosomes that are known for their instability (reviewed in [18]). Aneuploidy, defined as a change in the number of chromosome(s) or large portion of a chromosome, is well tolerated in fungi, including C. albicans. Spontaneous aneuploidy can be found in populations of C. albicans cells at high frequencies, between c. $10^{-4}$ and $10^{-2}$, with a clear tendency to increase under external stresses, and seems to be a basic property of this microbe [19]. It has been demonstrated that any chromosome of C. albicans can become aneuploid [18]. Various aneuploidies introduce diversity...
in a population of cells by controlling vital physiological functions, such as, for example, utilization of different carbon and nitrogen sources [20].

**Specific aneuploidies of *C. albicans* Ch5 or Ch2 control adaptation to ECNs**

In earlier work, the Rustchenko group demonstrated that *C. albicans* employs reversible alterations of specific chromosomes to adapt for growth in the presence of toxic agents that kill cells or prevent cell propagation, including fluconazole, 5-fluoro-orotic acid, and the toxic sugar L-sorbose (Table 1) (reviewed in [18]). Interestingly, adaptation to utilize the secondary sugar D-arabinose also relied on specific alterations of two specific chromosomes [18,21]. Recently, similar experiments demonstrated that reversible aneuploidies of Ch5 or Ch2 can control adaptation to the ECN caspofungin (Table 1) [13,22].

Loss of one Ch5 results in reduced susceptibility to caspofungin (denoted Cas+). In the absence of caspofungin, spontaneous duplication of the remaining Ch5 reverts Cas− cells to the original (parental strain) susceptibility [13] (Table 1). Loss and reduplication of Ch5 also controls resistance to the toxic sugar sorbose (denoted Sou+) [24], which kills fungi via a mechanism similar to ECN drugs by inhibiting glucan synthase [25,26]. Interestingly, Ch5 monosomy also confers adaptation to the antifungal pyrimidine analog 5-fluorocytosin (denoted 5-Fl+), as well as increased susceptibility to theazole antifungal fluconazole and the polyene antifungal amphotericin B (denoted FluS and AmBS, respectively) [23] (Table 1). Another aneuploid state conferring a Cas+ phenotype involves duplication of the right arm of Ch5 to create an isochromosome with two right arms (iso-Ch5R) (Fig 2). Note that these cells carry one iso-Ch5R and one normal Ch5, resulting in three right arms and one left arm of Ch5. Importantly, spontaneous duplication of the remaining normal Ch5 reverts cells to caspofungin susceptibility [13]. In contrast, duplication of the left arm of Ch5 resulting in iso-Ch5L with two left arms confers decreased susceptibility to fluconazole, FluR (Fig 2), and is found in resistant isolates, as well as in vitro generated mutants [27]. Also, trisomy of Ch2 confers Cas+ combined with adaptation to hydroxyurea, Hu+[22]. Of note, genes residing on Ch2 responsible for Cas+ phenotype have not yet been identified. However, it is clear that the reversible duplication of Ch2, in contrast to reversible loss of one Ch5, implies that genes for positive regulation of the Cas+ phenotype exist on Ch2. It is of interest to this review that exposure of *C. albicans* cells to chemotherapeutic drug hydroxyurea results in an Hu+ phenotype due to Ch2 trisomy, similarly to exposure to caspofungin [22], the effect, which might contribute to development of *Candida* infection in chemotherapy-treated patient [28]. It is worth mentioning that occasionally a mutant adapts to sorbose via a large deletion within the right arm of Ch5,

| Chromosome | Alteration | Phenotype       | Refs   |
|------------|------------|----------------|--------|
| Ch5        | Monosomy   | Cas+, Sou+, 5-Fl+, Flu+, AmB+ | [13,23] |
|            | Approximately 395 kb truncation of the right arm adjacent to telomere | Cas+, Sou+ | [17] |
|            | Iso-Ch5R   | Cas+           | [13]   |
| Ch2        | Trisomy    | Cas+, Hu+      | [22]   |

The table indicates phenotypes compared to parental diploid strains. Cas+, 5-Fl+, and Hu+ designate reduced susceptibility to caspofungin, 5-fluorocytosin, and hydroxyurea, respectively. FluS or AmBS designate increased susceptibility to fluconazole or amphotericin B, respectively. Sou+ designates resistance to and utilization of toxic sugar L-sorbose.

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instead of the loss of an entire chromosome. Consistently, large truncations of the right arm of Ch5 confer both, Sou\textsuperscript{+} and Cas\textsuperscript{+}, phenotypes \cite{17,29} (Table 1, Fig 2).

**Complexity of regulation by aneuploidy**

Long-term studies of reversible aneuploidy in response to challenge by caspofungin or the toxic sugar sorbose as a model system for \textit{C. albicans} adaptation to ECNs have unraveled an astonishingly complex and multilayered regulation of the genes responsible for these phenotypes. These studies show that the rate of formation of (predominantly Ch5 monosomic) Sou\textsuperscript{+} mutants per viable cell per day increased from $10^{-6}$ at the initial time of detection to $10^{-2}$ after four days of incubation on selective media \cite{24}. These data suggest that nondisjunction of a Ch5 homologue can occur by different mechanisms and that preexisting and adaptive mutants occur by different processes; the latter ones possibly involving a nonmitotic mechanism. At least nine spatially separated, functionally redundant regions that negatively control resistance to sorbose were identified on Ch5 (Fig 2). These regions, which fall into two functionally redundant pathways I and II (Fig 2), bear no sequence similarity among themselves and four of them bear no similarity to any known sequence. The regions are thought to encompass \textit{CSU} (Control of Sorbose Utilization) genes for negative control of sorbose resistance, three of which \textit{CSU51}, \textit{CSU53}, and \textit{CSU57} were identified in three corresponding regions (Fig 2) \cite{29,31,32}. Most importantly, \textit{CSU51}, a putative transcription factor, negatively controls both ECN susceptibilities and sorbose resistance \cite{17}. It remains to be determined whether the two other known \textit{CSU}s are also implicated with ECNs. The fact that \textit{CSU51} performs this dual function is remarkable, as it opens the possibility that many of the unique \textit{CSU} regulators predicted to reside in different regions of Ch5 also have dual function. Furthermore, two genes, \textit{PGA4} and \textit{CHT2}, which participate in cell wall construction and encode negative regulators of only ECN susceptibilities, have also been identified (Fig 2) \cite{17}. However, the final number of either \textit{CSU}s or genes involved only in ECN susceptibility remains elusive.

It is tempting to explain the Cas\textsuperscript{+} phenotype resulting from Ch5 monosomy as due to diminished gene dose of multiple negative regulators scattered across this chromosome.
However, the fact that the formation of iso-Ch5R, resulting in three right arms versus one left arm of Ch5, also results in diminished ECN susceptibility in corresponding cells implies that an additional or more complicated scenario can be in play. The right arm of Ch5 carries two genes, MID1 and CNB1, which encode positive regulators of ECN susceptibility [34,35]. Therefore, it is possible that the effects of various Ch5 ploidies are due to a balance between negative and positive factors expressed from this chromosome. From this point of view, the loss of one Ch5 diminishes the action of both negative and positive regulators expressed from this chromosome; negative regulators overriding positive regulators. Whereas, in the condition of iso-Ch5R, the amplified positive regulators on the right arm of Ch5 override the action of negative regulators on this arm.

As an example, Ch5 contains two key genes of interest, TAC1, a positive regulator of CDR1 and CDR2 genes encoding fluconazole efflux pumps on Ch3, and ERG11, a target gene for fluconazole (Fig 2A) [27]. Thus, loss of one Ch5, diminishing the copy number of TAC1 and ERG11, likely leads to increased sensitivity to fluconazole via loss of activity of these efflux pumps and the fluconazole target. In addition, as loss of one Ch5 also increases sensitivity to amphotericin B (see Table 1), it may be expected that Ch5 also carries genes for positive regulation of susceptibility to this drug. On a contrary, negative regulator(s) of antifungal 5-fluorocytosin could be also expected to reside on Ch5, as cells with monosomic Ch5 acquire 5-Fl phenotype (see Table 1).

An additional level of regulation includes regulatory elements denoted ASU (Antisense regulators of Sorbose Utilization) that are embedded within CSUs in an antisense configuration (Fig 2). In respect to the CSU transcripts, the ASU long noncoding transcripts are completely overlapped by CSU transcripts, are in lesser amounts, and are inversely related. Presumably, ASU transcripts modulate CSU transcripts [31]. Some genes residing on aneuploid chromosomes are also controlled by transcriptional compensation for gene dosage, which keeps expression of select genes at or near the diploid level, irrespectively of chromosome ploidy [36,37]; MID1 and CNB1 exemplifying such genes (Fig 2) [13]. Indeed, widespread dosage compensation occurs across monosomic Ch5 and correlates with increased chromosome-wide acetylation of histone H4 [38]. This epigenetic feature involves the histone acetyltransferase complex NuA4, which could be a novel drug target to reduce the viability of resistant cells. On the other hand, decreased expression of some genes to the diploid level has been shown to occur within the trisomic Ch4/7 and correlates with increased acetylation of histone H3, but the histone acetyltransferases involved have not been identified [38].

Last, but not least, the C. albicans genome contains two genes, FKS2 and FKS3, which have considerable sequence identity with the key ECN resistance gene FKS1 but act as negative regulators of FKS1 [39]. While heterozygous deletion of the essential FKS1 gene results in increased ECN susceptibility, complete removal of either FKS2 or FKS3 results, in contrast, in decreased ECN susceptibility due to resultant overexpression of FKS1, leading to an increase in cell wall glucan. Other indications of the involvement of FKS2 and FKS3 in ECN susceptibility include wide variations in the expression levels of these genes relative to FKS1 in clinical resistant isolates, down-regulation in spontaneous laboratory mutants harboring FKS1 resistance mutations [40], as well as down-regulation in model mutants bearing monosomic Ch5 or iso-Ch5R but lacking FKS1 resistance mutations [13].

ECN susceptibility in C. albicans can be diminished by a limited number of distinct aneuploidies of Ch5 and Ch2, which is consistent with an earlier assumption that genes of C. albicans are distributed over chromosomes nonrandomly [29,41]. A number of genes and processes relevant to this control already have been elucidated and inform about much-needed potential drug targets. However, we are clearly at the beginning of an exciting journey into understanding the regulation of ECN drug susceptibility by chromosome aneuploidy, which
involves a complex interplay between ratio of negative and positive regulators on Ch5 and Ch2, the regulation of FKS genes residing outside Ch5 and Ch2, additional factors involved in the complex regulation of genes on aneuploid chromosomes, and still unidentified genes and features. A better understanding of the control exerted by aneuploidies will help to better understand evolution of ECN drug resistance and will facilitate the identification of new drug targets.

References

1. Almeida F, Rodrigues ML, Coelho C. The still underestimated problem of fungal diseases worldwide. Front Microbiol. 2019; 10:214. Epub 2019/02/28. https://doi.org/10.3389/fmicb.2019.00214 PMID: 30809215; PubMed Central PMCID: PMC6379264.

2. Shor E, Perlin DS. Coping with stress and the emergence of multidrug resistance in fungi. PLoS Pathog. 2015; 11(3):e1004668. Epub 2015/03/20. https://doi.org/10.1371/journal.ppat.1004668 PMID: 25790300; PubMed Central PMCID: PMC4366371.

3. Mayer FL, Kronstad JW. Disarming Fungal Pathogens: *Bacillus safensis* inhibits virulence factor production and biofilm formation by *Cryptococcus neoformans* and *Candida albicans*. MBio. 2017; 8(5). Epub 2017/10/05. https://doi.org/10.1128/mBio.01537-17 PMID: 28974618; PubMed Central PMCID: PMC5626971.

4. Butts A, Palmer GE, Rogers PD. Antifungal adjuvants: preserving and extending the antifungal arsenal. Virulence. 2017; 8(2):198–210. Epub 2016/07/28. https://doi.org/10.1080/21505594.2016.1216283 PMID: 27459018; PubMed Central PMCID: PMC5354161.

5. Park S, Kelly R, Kahn JN, Robles J, Hsu MJ, Register E, et al. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical *Candida* sp. isolates. Antimicrob Agents Chemother. 2005; 49(8):3264–73. Epub 2005/07/29. https://doi.org/10.1128/AAC.49.8.3264-3273.2005 PMID: 16048935; PubMed Central PMCID: PMC1196231.

6. Perlin DS. Mechanisms of echinocandin antifungal drug resistance. Ann N Y Acad Sci. 2015; 1354:1–11. https://doi.org/10.1111/nyas.12831 PMID: 26190298.

7. Espinel-Ingraffea A, Arendrup M, Cantoñ E, Cordoba S, Dannaoui E, García-Rodríguez J, et al. Multicenter study of method-dependent epidemiological cutoff values for detection of resistance in *Candida* spp. and *Aspergillus* spp. to amphotericin B and echinocandins for the eTest agar diffusion method. Antimicrob Agents Chemother. 2017; 61(1):e01792–16. https://doi.org/10.1128/AAC.01792-16 PMID: 27799206.

8. Matsumoto E, Boyken L, Tendolkar S, McDanel J, Castanheira M, Pfaller M, et al. Candidemia surveillance in Iowa: emergence of echinocandin resistance. Diagn Microbiol Infect Dis. 2014; 79(2):205–8. https://doi.org/10.1016/j.diagmicrobio.2014.02.016 PMID: 24666704.

9. Pfaller MA, Messer SA, Diekema DJ, Jones RN, Castanheira M. Use of micafungin as a surrogate marker to predict susceptibility and resistance to caspofungin among 3,764 clinical isolates of *Candida* by use of CLSI methods and interpretive criteria. J Clin Microbiol. 2014; 52(1):108–14. https://doi.org/10.1128/JCM.02481-13 PMID: 24153129.

10. Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M. Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic and temporal trends of antifungal resistance. J Clin Microbiol. 2013; 51(8):2571–81. https://doi.org/10.1128/JCM.00308-13 PMID: 23720791.

11. Healey KR, Katiyar SK, Castanheira M, Pfaller MA, Edlind TD. *Candida glabrata* mutants demonstrating paradoxical reduced caspofungin susceptibility but increased micafungin susceptibility. Antimicrob Agents Chemother. 2011; 55(8):3947–9. https://doi.org/10.1128/AAC.00044-11 PMID: 21628537.

12. Locke JB, Almaguer AL, Zuill DE, Bartizal K. Characterization of in vitro resistance development to the novel echinocandin CD101 in *Candida* Species. Antimicrob Agents Chemother. 2016; 60(10):6100–7. Epub 2016/08/03. https://doi.org/10.1128/AAC.00620-16 PMID: 27480852; PubMed Central PMCID: PMC5038289.

13. Yang F, Zhang L, Wakabayashi H, Myers J, Jiang Y, Cao Y, et al. Tolerance to caspofungin in *Candida albicans* is associated with at least three distinctive mechanisms that govern expression of FKS genes and cell wall remodeling. Antimicrob Agents Chemother. 2017; 61(5):e0071-17. https://doi.org/10.1128/AAC.00071-17 PMID: 28223394.

14. Singh-Babak SD, Babak T, Diezmann S, Hill JA, Xie JL, Chen Y-L, et al. Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. PLoS Pathog. 2012; 8(5):e1002718. https://doi.org/10.1371/journal.ppat.1002718 PMID: 22615574.


Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in drug-resistant
Candida albicans. J Bacteriol. 1990; 172 (3):1276–83. Epub 1990/03/01. https://doi.org/10.1128/jb.172.3.1276-1283.1990 PMID: 2407719; PubMed Central PMCID: PMC208595.

Rustchenko EP, Howard DH, Sherman F. Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of Candida albicans. J Bacteriol. 1997; 176(11):3231–41. Epub 1994/06/01. https://doi.org/10.1128/jb.176.11.3231-3241.1994 PMID: 8195078; PubMed Central PMCID: PMC205493.

Yang F, Teoh F, Tan ASM, Cao Y, Pavelka N, Berman J. Aneuploidy enables cross-adaptation to unrelated drugs. Mol Biol Evol. 2019; 36(8):1768–82. https://doi.org/10.1093/molbev/msz104 PMID: 31026938.

Yang F, Kravets A, Bethelody G, Welle S, Rustchenko E. Chromosome 5 monosomy of Candida albicans controls susceptibility to various toxic agents, including major antifungals. Antimicrob Agents Chemother. 2008; 68(3):624–41. Epub 2008/03/28. https://doi.org/10.1128/AAC.00516-13 PMID: 18363649.

Crocken B, Tatum EL. The effect of sorbose on metabolism and morphology of Neurospora. Biochim Biophys Acta. 1968; 156(1):1–8. Epub 1968/02/01. https://doi.org/10.1016/0304-4165(68)90097-4 PMID: 5645744.

Elorza MV, Arst HN Jr. Sorbose resistant mutants of Aspergillus nidulans. Mol Gen Genet. 1971; 111(2):185–93. Epub 1971/01/01. https://doi.org/10.1007/BF00267792 PMID: 5564468.

Selmecki A, Gerami-Nejad M, Paulson C, Forche A, Berman J. An isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1. Mol Microbiol. 2008; 68(3):624–41. Epub 2008/03/28. https://doi.org/10.1111/j.1365-2958.2008.06176.x PMID: 18363649.

Teoh F, Pavelka N. How chemotherapy increases the risk of systemic Candidiasis in cancer patients: Current Paradigm and Future Directions. Pathogens. 2016; 5(1). Epub 2016/01/20. https://doi.org/10.1101/journal.pgen.1000705 PMID: 19876375; PubMed Central PMCID: PMC2760147.

Ahmad A, Kravets A, Rustchenko E. Transcriptional regulatory circuitries in the human pathogen Candida albicans involving sense-antisense interactions. Genetics. 2012; 190(2):537–47. https://doi.org/10.1534/genetics.111.136267 PMID: 22135347.

Reddy PK, Pullepu D, Dhabalia U, Udaya Prakash SM, Kabir MA. CSU57 encodes a novel repressor of sorbose utilization in opportunistic human fungal pathogen Candida albicans. Yeast. 2020. Epub 2020/11/13. https://doi.org/10.1002/yea.3537 PMID: 33179314.

Selmecki AM, Delmage K, Coven LE, Anderson JB, Berman J. Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. PLoS Genet. 2009; 5(10): e1000705. Epub 2009/10/31. https://doi.org/10.1371/journal.pgen.1000705 PMID: 19876375; PubMed Central PMCID: PMC2760147.

Plaine A, Walker L, Da Costa G, Mora-Montes HM, McKinnon A, Gow NA, et al. Functional analysis of Candida albicans GPI-anchored proteins: roles in cell wall integrity and caspofungin sensitivity. Fungal
35. Singh SD, Robbins N, Zaas AK, Schell WA, Perfect JR, Cowen LE. Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. PLoS Pathog. 2009; 5(7):e1000532. Epub 2009/08/04. https://doi.org/10.1371/journal.ppat.1000532 PMID: 19649312; PubMed Central PMCID:PMC2712069.

36. Kravets A, Qin H, Ahmad A, Bethlendy G, Gao Q, Rustchenko E. Widespread occurrence of dosage compensation in *Candida albicans*. PLoS ONE. 2010; 5(6):e10856. https://doi.org/10.1371/journal.pone.0010856 PMID: 20552010

37. Tucker C, Bhattacharya S, Wakabayashi H, Bellaousov S, Kravets A, Welle SL, et al. Transcriptional regulation on aneuploid chromosomes in diverse *Candida albicans* mutants. Sci Rep. 2018; 8(1):1630. https://doi.org/10.1038/s41598-018-20106-9 PMID: 29374238

38. Wakabayashi H, Tucker C, Bethlendy G, Kravets A, Welle SL, Bulger M, et al. NuA4 histone acetyltransferase activity is required for H4 acetylation on a dosage-compensated monosomic chromosome that confers resistance to fungal toxins. Epigenetics Chromatin. 2017; 10(1):49. https://doi.org/10.1186/s13072-017-0156-y PMID: 29061172

39. Suwunnakorn S, Wakabayashi H, Kordalewska M, Perlin DS, Rustchenko E. *FKS2* and *FKS3* genes of opportunistic human pathogen *Candida albicans* influence echinocandin susceptibility. Antimicrob Agents Chemother. 2018; 62(4):e02299–17. https://doi.org/10.1128/AAC.02299-17 PMID: 29358288

40. Douglas CM, D'Ippolito JA, Shei GJ, Meinz M, Onishi J, Marrinan JA, et al. Identification of the *FKS1* gene of *Candida albicans* as the essential target of 1,3-beta-D-glucan synthase inhibitors. Antimicrob Agents Chemother. 1997; 41(11):2471–9. Epub 1997/11/26. https://doi.org/10.1128/AAC.41.11.2471 PMID: 9371352; PubMed Central PMCID:PMC164147.

41. Perepnikhatka V, Fischer FJ, Niimi M, Baker RA, Cannon RD, Wang YK, et al. Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. J Bacteriol. 1999; 181(13):4041–9. Epub 1999/06/29. https://doi.org/10.1128/JB.181.13.4041-4049.1999 PMID: 10363973; PubMed Central PMCID:PMC93695.