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Hot topics in antifungal susceptibility testing: A new drug, a bad bug, sweeping caspofungin testing under the rug, and solving the ECV shrug

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Abstract

There are several new hot topics in antifungals and antifungal susceptibility testing. In this review, four topics of general interest to the clinical microbiology community are discussed. The first topic is the introduction of isavuconazole, a new triazole approved for clinical use in the US. The second is triazole resistance in *Aspergillus fumigatus* isolates. A specific set of mutations are being found with greater frequency in isolates globally, including the US. The third topic of interest is a word of caution about antifungal susceptibility testing for caspofungin in *Candida* isolates; some laboratories have reported susceptible isolates with high MIC values that would be interpreted as resistant. The final topic is an introduction to epidemiological cutoff values and their use in the clinical mycology laboratory.

Introduction

Antifungal susceptibility testing is not generally a topic at the top of most agendas in the clinical microbiology laboratory. Many laboratories do not offer this testing because of the expertise required and infrequency with which it is requested. With that in mind, it is prudent that a periodic review of what is new in antifungal susceptibility testing be provided. Four topics are currently being discussed in antifungal susceptibility testing laboratories. The first topic is the introduction of isavuconazole, a new triazole approved for clinical use in the US. The second is a new set of mutations within *Aspergillus fumigatus* isolates that confer resistance to azole drugs. These mutations are being found with greater frequency in isolates globally, including the US. The third topic of interest is the pitfalls of performing antifungal susceptibility testing for caspofungin in *Candida* isolates. Some laboratories report high MIC values that can be interpreted as resistant. The final topic is an introduction to epidemiological cutoff values and their use in the clinical mycology laboratory.

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Isavuconazole: A new drug

Compared to the number of antimicrobials available for the treatment of bacterial infections, the proverbial “cupboard” of antifungal agents is quite bare. Three classes of antifungal agents are available for the treatment of invasive infections: polyenes (amphotericin B), echinocandins (caspofungin, micafungin, and anidulafungin), and azoles (fluconazole, itraconazole, voriconazole and posaconazole). For primary therapy of mold infections only amphotericin B, itraconazole, voriconazole, and posaconazole are considered. For the first time since 2006 when posaconazole was released, the US Food and Drug Administration has approved the use of a new mold-active antifungal agent, the extended-spectrum triazole isavuconazole (trade name CRESEMBA®). Isavuconazole has been approved for the treatment of invasive aspergillosis, and it is also the first antifungal agent specifically indicated for the treatment of invasive mucormycosis. Isavuconazole is water soluble, eliminating the need for potentially toxic diluents, and it is available in both IV and oral formulations with no requirement to be taken with food for increased absorption. Like the other triazoles, isavuconazole is a lanosterol 14α demethylase inhibitor which blocks ergosterol production in fungi, leading to the accumulation of toxic sterol precursors and eventually to cell death (1).

In a recent phase 3 randomized double-blind trial of invasive aspergillosis, isavuconazole was compared directly to the currently recommended treatment for invasive aspergillosis, voriconazole (2). At the end of the trial, isavuconazole was found to be non-inferior to voriconazole. Trial patients who received isavuconazole also had significantly fewer drug-related adverse events and a lower rate of discontinuation of treatment due to those adverse events than those patients who received voriconazole (2).

Several studies have looked at the in vitro effectiveness of isavuconazole against Mucormycetes (3, 4). In one interesting study the authors performed in vitro susceptibility testing on 70 isolates of Mucorales. The in vitro activity of isavuconazole was species dependent with good activity against isolates of *Lichtheimia*, *Rhizomucor*, and *Rhizopus*, but higher MICs against *Mucor circinelloides* sensu lato. In lieu of outcome data, the authors compared the MIC values of the Mucorales isolates to the wild type range of MIC values for *Aspergillus fumigatus* (in other words, the epidemiological cutoff value, discussed below) with the hypothesis that if *A. fumigatus* isolates could be treated at that MIC value, so could Mucorales isolates. They called isolates with MICs in this range “potentially susceptible” (pot-S). They found that 80–100% of the isolates of *Lichtheimia*, *Rhizomucor* and *Rhizopus* were pot-S while only 11% of *Mucor circinelloides* isolates were pot-S (3).

Isavuconazole has good in vitro activity against many species of yeast (5–10). It shows significant activity against *Candida* species, *Cryptococcus neoformans* and *C. gattii*, *Rhodotorula*, *Saccharomyces*, and *Trichosporon*. There is currently a phase 3 clinical trial underway to study outcomes in patients with candidemia who are treated with isavuconazole. In vitro MIC values against non-*Aspergillus*, non-Mucormycete molds are variable and depend on both the species and the isolate (6, 11). More data are necessary before the clinical effectiveness of isavuconazole against these other molds can be determined.

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A. fumigatus: A bad bug

Aspergillosis can be a life threatening infection in patients with underlying health conditions and is most frequently caused by *A. fumigatus* (12). Fortunately, effective therapy has been available for more than a decade in the form of the triazole class of antifungals. As mentioned above, these antifungals target the ergosterol pathway involved in cell membrane synthesis, specifically the protein Erg11 which is encoded by the gene *cyp51A*. As this class of antifungal drug is also well tolerated by the patient and is available in both oral and intravenous formulations, it represents an ideal therapy for invasive fungal infections and one of these antifungals, voriconazole, is recommended as primary therapy for aspergillosis (13–15). Unfortunately, resistance to the azoles has been detected in clinical isolates of *A. fumigatus* from various parts of the world, threatening the future use of this primary antifungal agent.

The first reports of azole resistant *A. fumigatus* came from Europe although two of the isolates were from US patients (16–18). These early studies indicated that resistance was sporadic, rates of azole resistance were approximately 1.7%, and therefore azole resistance was still considered an infrequent although troubling occurrence (19). Often the patients involved had chronic aspergillus infections and had been undergoing azole therapy for an extended period of time, an indication that resistance had evolved under the selective pressure of their antifungal regimen. This acquired resistance is in contrast to intrinsic antifungal resistance observed in other fungal species, such as fluconazole resistance in *Candida krusei.* Investigation into the mechanisms underlying this azole resistance implicated multiple gene mutations within the drug target *cyp51A*. Clinically relevant mutations were found in the codons for multiple amino acids including G54 (read as glycine at amino acid number 54), P216, F219, M220, G138, Y431, and G448 (20). The majority of these mutations alter the antifungal target in such a way that the drug is no longer able to bind efficiently and antifungal activity is lost.

More recently, studies have indicated that the epidemiology of azole resistance in *A. fumigatus* has changed. Patients who are naïve to azole therapy have presented with azole-resistant aspergillosis with more frequency and the overall rates of azole resistance have increased dramatically (21, 22). This change was highlighted first by a 2007 report from the Netherlands which described an alarming rate of increased resistance and called for international surveillance into this potential health concern (23). Additional studies have reported detection of triazole resistance in *A. fumigatus* from other parts of the world including Australia, Europe, Asia, the Middle East, Africa, and South America (24–30). The overwhelming majority of these cases were caused by two different *cyp51A* mutations called TR34/L98H and TR46/Y121F/T289A. The TR34/L98H mutation is comprised of a 34 base pair tandem repeat in the *cyp51A* promoter region and a L98H (Lysine 98 to histidine) polymorphism within the Cyp51A amino acid sequence. Similarly, TR46/Y121F/T289A is comprised of a 46 base pair tandem repeat in the *cyp51A* promoter region and two polymorphisms within the Cyp51A amino acid sequence. These two mutations confer cross resistance to multiple azoles (31).

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There is evidence that the TR34/L98H and TR46/Y121F/T289A mutations have an environmental origin (32, 33). *Aspergillus* species are ubiquitous in nature and grow well in soil, around decomposing plants, and in areas of crop production (12). When azole fungicides are applied to protect agricultural plants from fungal pathogens, *A. fumigatus* is also exposed, providing opportunity for this pathogen to undergo genetic changes to overcome the stress caused by these fungicides. This is similar to the selection for resistance that occurs with drug exposure in a patient. Evidence for environmental selection includes the finding of this mutation in azole resistant *A. fumigatus* isolates cultured from soil and cultured from azole-naïve patients (28–30).

The TR34/L98H and TR46/Y121F/T289A mutations have only recently been identified within the US. In a study of 1,026 *A. fumigatus* isolates collected from across the US between 2011 and 2013 neither of these mutations was detected in any isolates (34). However, two recent reports showed that two isolates with TR34/L98H were collected from patients in Pennsylvania in 2010 and 2012 and isolates with TR46 Y121F/T289A were collected from patients in Arizona in 2008 and Michigan in 2014 (35, 36). This alarming discovery signifies that this emerging health problem is no longer only a concern in other parts of the world but in the US as well.

In the US, antifungal breakpoints to molds have not been determined, so resistance to a triazole antifungal is defined as an MIC that exceeds the established epidemiological cutoff value (see below). Elevated MICs are associated with poorer patient outcomes and complicate patient management by restricting therapeutic options. However, antifungal susceptibility testing is not often routinely performed in clinical microbiology laboratories and sending out for susceptibility testing results in long turn-around-times. When testing is performed, standard procedures require positive fungal culture, which may be difficult to obtain. This compounds the challenge of surveillance and resistance detection, and perhaps contributes to underrepresented true rates of azole resistant *A. fumigatus*.

Both environmental- and patient-based azole resistance in *A. fumigatus* continue to evolve globally as a public health challenge, a fact which is underscored by the remarkable shift in rates of resistance which has occurred in recent years. Moreover, the number of drugs in the development pipeline are few. In order to preserve the utility of available therapies, it is vital that we understand the evolution of azole resistance and assess its prevalence within the clinical population. To this end, the Centers for Disease Control and Prevention is conducting surveillance for azole resistant *A. fumigatus*. More information about this surveillance can be found here: http://www.cdc.gov/fungal/diseases/aspergillosis/index.html.

Sweeping caspofungin testing under the rug

The echinocandins are the newest class of antifungal agents and they are considered first line therapy for infections with *Candida* species (37, 38). There are three echinocandins available for clinical use: caspofungin, micafungin and anidulafungin. The Clinical and Laboratory Standards Institute (CLSI) has established breakpoints for resistance for all three echinocandins against *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, and *C. guilliermondii* (39). However, when using broth microdilution, some laboratories

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have experienced caspofungin MIC values that are higher than other laboratories and give the impression that most of the *Candida* isolates tested are caspofungin resistant (40, 41). This is not seen when testing the same isolates with micafungin or anidulafungin. Although some studies have attempted to determine the cause of this discrepancy such as testing different lots of caspofungin and using different types of microtiter plates, the overall cause has remained elusive (42). This phenomenon is an in vitro artifact and has no bearing on the clinical treatment of patients: there is no evidence that an isolate can be resistant to one echinocandin but susceptible to another. However, due to the interlaboratory variability when testing with caspofungin the European Committee on Antimicrobial Susceptibility Testing declined to establish breakpoints for caspofungin (43).

There are several alternatives to using microbroth dilution testing for caspofungin resistance. The first is to use micafungin or anidulafungin as a surrogate for testing against caspofungin. Surrogate testing is often used in susceptibility testing for bacteria and two studies show that it should be successful for *Candida* and the echinocandins as well (44, 45). Another alternative would be to perform susceptibility testing using the YeastOne® panel. While YeastOne® does not strictly follow the protocols outlined in the CLSI guidelines for broth microdilution susceptibility testing, the phenomenon of higher than normal caspofungin MIC values is not seen when testing using the YeastOne® panel (46, 47). In contrast, there is some evidence that this phenomenon may also apply to testing using Etest® so this cannot be recommended as an alternative (48). Given that there are viable alternatives, microbroth dilution susceptibility testing of *Candida* against caspofungin as outlined in CLSI M27-A3 is not currently recommended.

Solving the ECV shrug

When confronted with the concept of epidemiological cutoff values (ECVs) many lab directors and clinicians, shrug their shoulders and say “I don’t know what to do with these!” In the next few paragraphs we will introduce the concept of ECVs and explain why they are needed and how they should be used. This introduction will hopefully prepare directors, clinicians, and technologists for their use with fungi.

Why don’t we just keep establishing breakpoints?

In order to establish a breakpoint for any fungus/antifungal combination a number of things are needed: the MIC distribution of a large number of isolates, the pharmacokinetics/pharmacodynamics of the drug, and patient outcome data for a patient population treated with a specific dose of that antifungal. The MIC distribution data is the easiest to obtain, simply requiring testing of multiple isolates of the species against the drug of interest and recording the distribution of values. The pharmacokinetics/pharmacodynamics are not as easy. They can be determined using an animal model of infection, using healthy volunteers, or during intensive monitoring of a patient population during treatment. All of these are invasive, costly, and difficult with some of the rare fungal species that might only be seen once a year or less in any given institution. The last piece of necessary information for establishing a breakpoint is clinical outcome data. In general, this data is generated during a controlled clinical trial. Clinical trials are established in a relatively small number of

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institutions, and for many fungus/antifungal combinations there would simply not be enough cases to justify the effort and the tremendous cost. Outcome data can be generated outside of a clinical trial, but without patient population and treatment controls in place, the usefulness of this data is suspect. What all of this means is that there will probably never be any breakpoints for many fungus/antifungal combinations.

What do we do without breakpoints?

Without the benefit of breakpoints, clinicians rely on published case reports and institutional knowledge when deciding how to treat a particular patient. One of the central questions around treatment is what to do when a patient is failing therapy. Is the failure due to host factors or is the failure due to the drug not being effective against the fungus? How do we know if the isolate is resistant when there are no breakpoints to establish a definition for resistance? Often a clinician will ask for susceptibility testing, but the result is a set of numbers with no interpretive criteria. While the definition for resistance (i.e. the breakpoint) may not be available, there is a way to establish whether a particular isolate is wild type (i.e. without an acquired mechanism of resistance) in terms of susceptibility to a given antifungal agent and that is by comparing the MIC value of the isolate to the ECV.

What is an ECV?

An ECV is an MIC value that defines the upper limit of the wild type distribution of MIC values for any particular fungus/antifungal combination. An ECV indicates when an MIC value for a particular fungus/antifungal combination either looks like a “normal” (wild type) MIC value or it looks like a value that is higher than normal (non-wild type). An MIC value that is higher than normal may indicate antifungal resistance.

To establish an epidemiological cutoff value all that is needed is the distribution of MIC values for a particular fungus/antifungal combination. In the new CLSI document M57 the data requirement for establishing an ECV is 100 individual data points (fungus/antifungal MIC values) collected from at least three different laboratories, and generated using the established CLSI criteria (CLSI, in preparation). The endpoint for the wild type MIC distribution is established using a statistical algorithm that defines the endpoint encompassing 97.5% of the “modeled” distribution. The algorithm is not a straight $MIC_{97.5}$; in other words, it doesn’t just draw a line at the MIC under which 97.5% of the values lie. The algorithm gives higher weight to the values around the modal MIC (the MIC value with the highest number of isolates) so that having isolates that are not wild type in the data set does not skew the data to a higher MIC value (49).

For species with known mechanisms of antifungal resistance, the ECV can be tested by plotting the distribution of isolates that are known to be wild type against those that have a known mechanism of resistance. Figure 1 shows the distribution of *Candida glabrata* isolates that are known to be susceptible to micafungin against those known to have *FKS* mutation-mediated resistance to micafungin. While 1.7% of the wild type isolates have an MIC value above the ECV (determined to be 0.03 μg/ml (50)), no isolates with known mechanisms of resistance have an MIC value that falls below the ECV. It is possible that some of the wild type isolates with MIC values above the ECV have an unknown mechanism of resistance

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and are not truly wild type, but in any case, using the established ECV only 1.7% of the susceptible isolates are defined as non-wild type, and none of the resistant isolates are defined as wild type.

ECVs have been generated for a number of fungus/antifungal combinations using surveillance isolates as well as using combined data sets from multiple laboratories (50–62). While these ECVs are useful and quite often accurate, they were not established using a single set of defined criteria. The goal of CLSI document M-57 is to create a defined set of criteria for the establishment of fungal ECVs as well as to generate and publish ECV values for fungus/antifungal combinations for which there are no current breakpoints. This will include yeasts as well as molds.

How should ECVs be used?

Epidemiological cutoff values were not established as a replacement for breakpoints. However, they can be a useful tool in clinical decision making. In a laboratory information system (LIS) report, the MIC of a given fungus/antifungal combination can be provided to the clinician along with the ECV and a note that no breakpoints or interpretive criteria exist. If the MIC is above the ECV a comment can be added to the LIS report that the MIC is above the wild type MIC for that species and the isolate may have an acquired mechanism of resistance. The clinician will still be dependent upon institutional knowledge of how to typically treat the species in question, but MIC value in relation to the ECV may be another helpful piece of information, especially when the patient is not responding to therapy. An ECV is not a panacea for a lack of an established breakpoint, but it is an available tool that may assist in decision making for use of a particular antifungal agent against a particular isolate.

Summary

While changes in the clinical mycology laboratory do not happen at the same rate as changes in the bacteriology laboratory, there are changes in protocols, new antifungals in the armamentarium, and new resistance mechanisms appearing. Laboratories should be aware that clinicians may request susceptibility testing against the newest antifungal, isavuconazole. Testing may be requested to confirm or rule out triazole resistance in *Aspergillus fumigatus* isolates. And finally, new data are forthcoming in the form of ECVs that will allow the inclusion of some interpretive criteria for fungus/antifungal combinations for which there are no breakpoints.

References

1. Falci DR, Pasqualotto AC. Profile of isavuconazole and its potential in the treatment of severe invasive fungal infections. Infection and drug resistance. 2013; 6:163–74. [PubMed: 24187505]
2. Maertens JA, Raad, Marr KA, Patterson TF, Kontoyiannis DP, Cornely OA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by *Aspergillus* and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. Lancet (London, England). 2015 Dec 9.
3. Arendrup MC, Jensen RH, Meletiadis J. In vitro activity of isavuconazole and comparators against clinical isolates of the Mucorales order. Antimicrobial agents and chemotherapy. 2015 Oct 5.

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4. Verweij PE, Gonzalez GM, Wiedrhold NP, Lass-Florl C, Warn P, Heep M, et al. In vitro antifungal activity of isavuconazole against 345 mucorales isolates collected at study centers in eight countries. Journal of chemotherapy (Florence, Italy). 2009 Jun; 21(3):272–81.
5. Thompson GR 3rd, Wiederhold NP, Sutton DA, Fothergill A, Patterson TF. In vitro activity of isavuconazole against *Trichosporon, Rhodotorula, Geotrichum, Saccharomyces* and *Pichia* species. The Journal of antimicrobial chemotherapy. 2009 Jul; 64(1):79–83. [PubMed: 19406849]
6. Pfaller MA, Messer SA, Rhomberg PR, Jones RN, Castanheira M. In vitro activities of isavuconazole and comparator antifungal agents tested against a global collection of opportunistic yeasts and molds. Journal of clinical microbiology. 2013 Aug; 51(8):2608–16. [PubMed: 23740727]
7. Guinea J, Recio S, Escribano P, Pelaez T, Gama B, Bouza E. In vitro antifungal activities of isavuconazole and comparators against rare yeast pathogens. Antimicrobial agents and chemotherapy. 2010 Sep; 54(9):4012–5. [PubMed: 20566770]
8. Castanheira M, Messer SA, Rhomberg PR, Dietrich RR, Jones RN, Pfaller MA. Isavuconazole and nine comparator antifungal susceptibility profiles for common and uncommon *Candida* species collected in 2012: application of new CLSI clinical breakpoints and epidemiological cutoff values. Mycopathologia. 2014 Aug; 178(1–2):1–9. [PubMed: 24952015]
9. Illnait-Zaragozi MT, Martinez GF, Curfs-Breuker I, Fernandez CM, Boekhout T, Meis JF. In Vitro activity of the new azole isavuconazole (BAL4815) compared with six other antifungal agents against 162 *Cryptococcus neoformans* isolates from Cuba. Antimicrobial agents and chemotherapy. 2008 Apr; 52(4):1580–2. [PubMed: 18212095]
10. Espinel-Ingroff A, Chowdhary A, Gonzalez GM, Guinea J, Hagen F, Meis JF, et al. Multicenter study of isavuconazole MIC distributions and epidemiological cutoff values for the *Cryptococcus neoformans-Cryptococcus gattii* species complex using the CLSI M27-A3 broth microdilution method. Antimicrobial agents and chemotherapy. 2015 Jan; 59(1):666–8. [PubMed: 25313209]
11. Gonzalez GM. In vitro activities of isavuconazole against opportunistic filamentous and dimorphic fungi. Medical mycology. 2009 Feb; 47(1):71–6. [PubMed: 19101837]
12. Segal BH. *Aspergillosis*. N Engl J Med. 2009 Apr 30; 360(18):1870–84. [PubMed: 19403905]
13. Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. Int J Microbiol. 2012; 2012:713687. [PubMed: 22187560]
14. Sheehan DJ, Hitchcock CA, Sibley CM. Current and emerging azole antifungal agents. Clin Microbiol Rev. 1999 Jan; 12(1):40–79. [PubMed: 9880474]
15. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2008 Feb 1; 46(3):327–60. [PubMed: 18177225]
16. Chryssanthou E. In vitro susceptibility of respiratory isolates of *Aspergillus* species to itraconazole and amphotericin B. acquired resistance to itraconazole. Scand J Infect Dis. 1997; 29(5):509–12. [PubMed: 9435042]
17. Dannaoui E, Borel E, Monier MF, Piens MA, Picot S, Persat F. Acquired itraconazole resistance in *Aspergillus fumigatus*. J Antimicrob Chemother. 2001 Mar; 47(3):333–40. [PubMed: 11222566]
18. Mellado E, Garcia-Effron G, Alcazar-Fuoli L, Melchers WJ, Verweij PE, Cuenca-Estrella M, et al. A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of cyp51A alterations. Antimicrobial agents and chemotherapy. 2007 Jun; 51(6):1897–904. [PubMed: 17371828]
19. Snelders E, van der Lee HA, Kuijpers J, Rijs AJ, Varga J, Samson RA, et al. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. PLoS medicine. 2008 Nov 11.5(11):e219. [PubMed: 18998768]
20. Verweij PE, Chowdhary A, Melchers WJ, Meis JF. Azole Resistance in *Aspergillus fumigatus*: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles? Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2016 Feb 1; 62(3):362–8. [PubMed: 26486705]
21. Bueid A, Howard SJ, Moore CB, Richardson MD, Harrison E, Bowyer P, et al. Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. The Journal of antimicrobial chemotherapy. 2010 Oct; 65(10):2116–8. [PubMed: 20729241]

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22. Verweij PE, Te Dorsthorst DT, Rijs AJ, De Vries-Hospers HG, Meis JF. Nationwide survey of in vitro activities of itraconazole and voriconazole against clinical *Aspergillus fumigatus* isolates cultured between 1945 and 1998. J Clin Microbiol. 2002 Jul; 40(7):2648–50. [PubMed: 12089298]
23. Verweij PE, Mellado E, Melchers WJ. Multiple-triazole-resistant aspergillosis. The New England journal of medicine. 2007 Apr 5; 356(14):1481–3.
24. Lockhart SR, Frade JP, Etienne KA, Pfaller MA, Diekema DJ, Balajee SA. Azole resistance in *Aspergillus fumigatus* isolates from the ARTEMIS global surveillance study is primarily due to the TR/L98H mutation in the cyp51A gene. Antimicrobial agents and chemotherapy. 2011 Sep; 55(9): 4465–8. [PubMed: 21690285]
25. Kidd SE, Goeman E, Meis JF, Slavin MA, Verweij PE. Multi-triazole-resistant *Aspergillus fumigatus* infections in Australia. Mycoses. 2015 Jun; 58(6):350–5. [PubMed: 25885568]
26. Chowdhary A, Kathuria S, Randhawa HS, Gaur SN, Klaassen CH, Meis JF. Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the cyp51A gene in India. The Journal of antimicrobial chemotherapy. 2012 Feb; 67(2):362–6. [PubMed: 22028200]
27. Seyedmousavi S, Hashemi SJ, Zibafar E, Zoll J, Hedayati MT, Mouton JW, et al. Azole-resistant *Aspergillus fumigatus*, Iran. Emerging infectious diseases. 2013 May; 19(5):832–4. [PubMed: 23697829]
28. Le Pape P, Lavergne RA, Morio F, Alvarez-Moreno C. Multiple Fungicide-Driven Alterations in Azole-Resistant *Aspergillus fumigatus*, Colombia, 2015. Emerging infectious diseases. 2016 Jan; 22(1):156–7. [PubMed: 26690795]
29. Chowdhary A, Sharma C, van den Boom M, Yntema JB, Hagen F, Verweij PE, et al. Multi-azole-resistant *Aspergillus fumigatus* in the environment in Tanzania. The Journal of antimicrobial chemotherapy. 2014 Nov; 69(11):2979–83. [PubMed: 25006238]
30. Ahmad S, Khan Z, Hagen F, Meis JF. Occurrence of triazole-resistant *Aspergillus fumigatus* with TR34/L98H mutations in outdoor and hospital environment in Kuwait. Environmental research. 2014 Aug.133:20–6. [PubMed: 24906064]
31. van der Linden JW, Camps SM, Kampinga GA, Arends JP, Debets-Ossenkopp YJ, Haas PJ, et al. Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2013 Aug; 57(4):513–20. [PubMed: 23667263]
32. Snelders E, Huis In 't Veld RA, Rijs AJ, Kema GH, Melchers WJ, Verweij PE. Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. Applied and environmental microbiology. 2009 Jun; 75(12):4053–7. [PubMed: 19376899]
33. Verweij PE, Snelders E, Kema GH, Mellado E, Melchers WJ. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? The Lancet Infectious diseases. 2009 Dec; 9(12):789–95. [PubMed: 19926038]
34. Pham CD, Reiss E, Hagen F, Meis JF, Lockhart SR. Passive surveillance for azole-resistant *Aspergillus fumigatus*, United States, 2011–2013. Emerging infectious diseases. 2014 Sep; 20(9): 1498–503. [PubMed: 25148217]
35. Wiederhold NP, Gil VG, Gutierrez F, Lindner JR, Albataineh MT, McCarthy DI, et al. First Detection of TR34 L98H and TR46 Y121F T289A Cyp51 Mutations in *Aspergillus fumigatus* Isolates in the United States. J Clin Microbiol. 2016 Jan; 54(1):168–71. [PubMed: 26491179]
36. Vazquez JA, Manavathu EK. Molecular Characterization of a Voriconazole-Resistant, Posaconazole-Susceptible *Aspergillus fumigatus* Isolate in a Lung Transplant Recipient in the United States. Antimicrobial agents and chemotherapy. 2015; 60(2):1129–33. [PubMed: 26574014]
37. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2016 Feb 15; 62(4):e1–e50. [PubMed: 26679628]
38. Perlin DS. Echinocandin resistance, susceptibility testing and prophylaxis: implications for patient management. Drugs. 2014 Sep; 74(14):1573–85. [PubMed: 25255923]

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39. Institute CaLS. M27-S4 Reference method for broth dilution antifungal susceptibility testing of yeasts; fourth informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
40. Espinel-Ingroff A, Arendrup MC, Pfaller MA, Bonfietti LX, Bustamante B, Canton E, et al. Interlaboratory variability of Caspofungin MICs for *Candida* spp. Using CLSI and EUCAST methods: should the clinical laboratory be testing this agent? Antimicrobial agents and chemotherapy. 2013 Dec; 57(12):5836–42. [PubMed: 24018263]
41. Shields RK, Nguyen MH, Press EG, Kwa AL, Cheng S, Du C, et al. The presence of an FKS mutation rather than MIC is an independent risk factor for failure of echinocandin therapy among patients with invasive candidiasis due to *Candida glabrata.* Antimicrobial agents and chemotherapy. 2012 Sep; 56(9):4862–9. [PubMed: 22751546]
42. Fothergill AW, McCarthy DI, Albataineh MT, Sanders C, McElmeel M, Wiederhold NP. The Effects of Treated vs. Untreated Polystyrene on Caspofungin In Vitro Activity against *Candida* Species. Journal of clinical microbiology. 2016 Jan 13.
43. Arendrup MC, Cuenca-Estrella M, Lass-Florl C, Hope WW. EUCAST technical note on *Candida* and micafungin, anidulafungin and fluconazole. Mycoses. 2014 Jun; 57(6):377–9. [PubMed: 24417759]
44. 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. Journal of clinical microbiology. 2014 Jan; 52(1):108–14. [PubMed: 24153129]
45. Pfaller MA, Diekema DJ, Jones RN, Castanheira M. Use of anidulafungin as a surrogate marker to predict susceptibility and resistance to caspofungin among 4,290 clinical isolates of *Candida* by using CLSI methods and interpretive criteria. Journal of clinical microbiology. 2014 Sep; 52(9): 3223–9. [PubMed: 24951808]
46. Eschenauer GA, Nguyen MH, Shoham S, Vazquez JA, Morris AJ, Pasculle WA, et al. Real-world experience with echinocandin MICs against *Candida* species in a multicenter study of hospitals that routinely perform susceptibility testing of bloodstream isolates. Antimicrobial agents and chemotherapy. 2014; 58(4):1897–906. [PubMed: 24395235]
47. Espinel-Ingroff A, Alvarez-Fernandez M, Canton E, Carver PL, Chen SC, Eschenauer G, et al. Multicenter study of epidemiological cutoff values and detection of resistance in *Candida* spp. to anidulafungin, caspofungin, and micafungin using the Sensititre YeastOne colorimetric method. Antimicrobial agents and chemotherapy. 2015 Nov; 59(11):6725–32. [PubMed: 26282428]
48. Arendrup MC, Pfaller MA. Caspofungin Etest susceptibility testing of Candida species: risk of misclassification of susceptible isolates of *C. glabrata* and *C. krusei* when adopting the revised CLSI caspofungin breakpoints. Antimicrobial agents and chemotherapy. 2012 Jul; 56(7):3965–8. [PubMed: 22564836]
49. Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2006 May; 12(5):418–25.
50. Pfaller MA, Espinel-Ingroff A, Bustamante B, Canton E, Diekema DJ, Fothergill A, et al. Multicenter study of anidulafungin and micafungin MIC distributions and epidemiological cutoff values for eight *Candida* species and the CLSI M27-A3 broth microdilution method. Antimicrobial agents and chemotherapy. 2014; 58(2):916–22. [PubMed: 24277027]
51. Espinel-Ingroff A, Aller AI, Canton E, Castanon-Olivares LR, Chowdhary A, Cordoba S, et al. *Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole. Antimicrobial agents and chemotherapy. 2012 Nov; 56(11):5898–906. [PubMed: 22948877]
52. Espinel-Ingroff A, Chowdhary A, Gonzalez GM, Lass-Florl C, Martin-Mazuelos E, Meis J, et al. Multicenter study of isavuconazole MIC distributions and epidemiological cutoff values for *Aspergillus* spp. for the CLSI M38-A2 broth microdilution method. Antimicrobial agents and chemotherapy. 2013 Aug; 57(8):3823–8. [PubMed: 23716059]

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53. Espinel-Ingroff A, Cuenca-Estrella M, Fothergill A, Fuller J, Ghannoum M, Johnson E, et al. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B and *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). Antimicrobial agents and chemotherapy. 2011 Nov; 55(11):5150–4. [PubMed: 21876047]
54. Espinel-Ingroff A, Diekema DJ, Fothergill A, Johnson E, Pelaez T, Pfaller MA, et al. Wild-type MIC distributions and epidemiological cutoff values for the triazoles and six *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). Journal of clinical microbiology. 2010 Sep; 48(9):3251–7. [PubMed: 20592159]
55. Espinel-Ingroff A, Pfaller MA, Bustamante B, Canton E, Fothergill A, Fuller J, et al. Multilaboratory study of epidemiological cutoff values for detection of resistance in eight *Candida* species to fluconazole, posaconazole, and voriconazole. Antimicrobial agents and chemotherapy. 2014; 58(4):2006–12. [PubMed: 24419346]
56. Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. Wild-type minimum effective concentration distributions and epidemiologic cutoff values for caspofungin and *Aspergillus* spp. as determined by Clinical and Laboratory Standards Institute broth microdilution methods. Diagnostic microbiology and infectious disease. 2010 May; 67(1):56–60. [PubMed: 20207095]
57. Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. Wild-type MIC distributions and epidemiological cutoff values for posaconazole and voriconazole and *Candida* spp. as determined by 24-hour CLSI broth microdilution. Journal of clinical microbiology. 2011 Feb; 49(2):630–7. [PubMed: 21159940]
58. Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, et al. Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. Journal of clinical microbiology. 2010 Jan; 48(1):52–6. [PubMed: 19923478]
59. Pfaller MA, Castanheira M, Diekema DJ, Messer SA, Jones RN. Wild-type MIC distributions and epidemiologic cutoff values for fluconazole, posaconazole, and voriconazole when testing *Cryptococcus neoformans* as determined by the CLSI broth microdilution method. Diagnostic microbiology and infectious disease. 2011 Nov; 71(3):252–9. [PubMed: 21917395]
60. Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN. Echinocandin and triazole antifungal susceptibility profiles for *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus fumigatus*: application of new CLSI clinical breakpoints and epidemiologic cutoff values to characterize resistance in the SENTRY Antimicrobial Surveillance Program (2009). Diagnostic microbiology and infectious disease. 2011 Jan; 69(1):45–50. [PubMed: 21146713]
61. Pfaller MA, Diekema DJ, Ghannoum MA, Rex JH, Alexander BD, Andes D, et al. Wild-type MIC distribution and epidemiological cutoff values for *Aspergillus fumigatus* and three triazoles as determined by the Clinical and Laboratory Standards Institute broth microdilution methods. Journal of clinical microbiology. 2009 Oct; 47(10):3142–6. [PubMed: 19692559]
62. Pfaller MA, Espinel-Ingroff A, Canton E, Castanheira M, Cuenca-Estrella M, Diekema DJ, et al. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and *Candida* spp. as determined by CLSI broth microdilution. Journal of clinical microbiology. 2012 Jun; 50(6):2040–6. [PubMed: 22461672]

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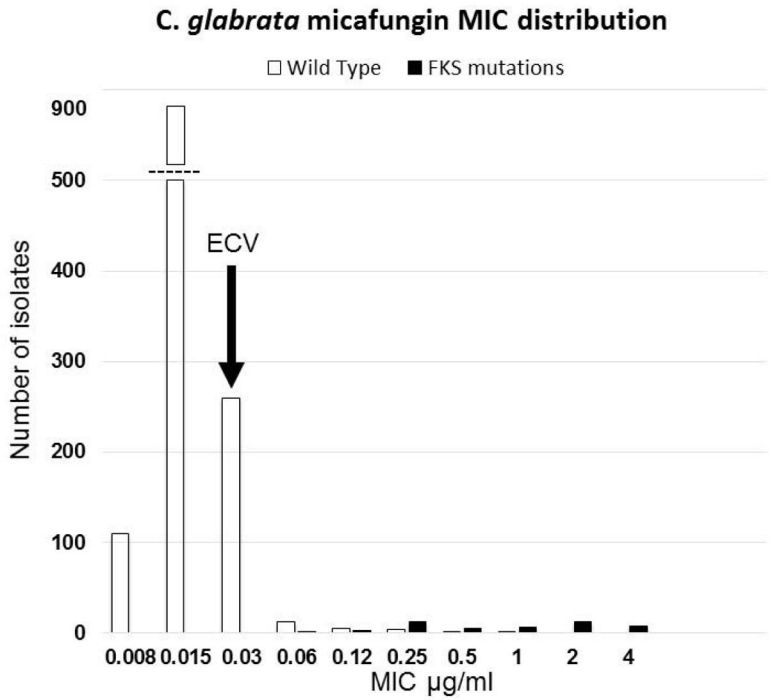

Figure 1.

Micafungin MIC distribution of 1,380 isolates of *C. glabrata*. The white bars represent wild type isolates, and the black bars represent isolates with *FKS* mutation-mediated resistance to micafungin. Note that the ECV (0.03 µg/ml; black arrow) accurately defines the cutoff for isolates with known mechanisms of resistance.