Chapter 2
Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods

Annette W. Fothergill

Abstract Antifungal susceptibility testing has become an important tool for physicians faced with making difficult treatment decisions regarding treatment of patients with fungal infections. The Clinical Laboratory and Standards Institute (CLSI) has approved methods for testing of both yeast and moulds. Testing may be accomplished via macrobroth, microbroth, or disk methods. In addition to CLSI methods, industry has provided a variety of both manual and automated systems for determining antifungal susceptibility for fungi. This, combined with an expanded list of interpretive data, has elevated antifungal susceptibility testing to a level of importance as a diagnostic test.

2.1 Introduction

Antifungal susceptibility testing (AST) has been a recognized diagnostic tool for over 20 years. Despite this, interpretation of the results and determination of how best to use these results continue to cause considerable confusion. Over the past two decades, antifungal susceptibility has undergone considerable change. AST has evolved from a nonstandardized procedure that generated results lacking clinical utility to a standardized procedure that is well controlled and that gives results physicians may use to assist with making tough clinical decisions. The Clinical and Laboratory Standards Institute (CLSI), formerly the NCCLS (National Committee on Clinical Laboratory Standards), has released four standard methods for antifungal susceptibility testing including M27-A3 [1] for macrobroth and microtiter yeast testing, M38-A2 [2] for microtiter mould testing, M44-A [3] for yeast disk diffusion testing, and M51-P [4] for mould disk diffusion testing.

A.W. Fothergill, M.A., M.B.A., MT(ASCP), CLS(NCA) (✉)
Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA
e-mail: fothergill@uthscsa.edu

G.S. Hall (ed.), *Interactions of Yeasts, Moulds, and Antifungal Agents: How to Detect Resistance*, DOI 10.1007/978-1-59745-134-5_2,

2.2 History

In 1985, the subcommittee on AST released its first report [5]. This document, M20-CR, Antifungal Susceptibility Testing: Committee Report, was compiled from responses to a questionnaire sent to hospitals and reference laboratories. This document indicated that AST was being conducted by approximately 20% of institutions that responded but on a rather small scale and that most of the sites conducting testing were utilizing some form of broth testing. Other methods in use included agar and disk diffusion methods. It was also noted that the method variability contributed to minimum inhibitory concentrations (MICs) that could not be reliably reproduced between institutions. Given these findings, the committee concluded that it was necessary and would be of use to the medical community to proceed toward a standardized method.

Based on an evaluation of findings, it was determined that the reference method should be a broth dilution method. Having chosen this starting point, several other parameters required investigation including inoculum preparation, test medium, incubation temperature, incubation duration, and criteria for endpoint determination. The first standard was published in 1992 [6] and was a macrobroth dilution method requiring 1-ml volumes in tubes. To avoid drug-medium interactions, RPMI-1640, a totally defined medium, was selected. Optimum incubation was determined to be 35°C for 48 or 72 h depending on species. The endpoint was defined as the lowest dilution that resulted in zero visible growth for amphotericin B or in an 80% reduction in turbidity as compared to the drug-free control tube for the azoles and 5-fluorocytosine.

Subsequent publications provided for a microtiter dilution method where parameters were the same but where endpoints were defined as zero visible growth for amphotericin B or a 50% reduction in turbidity as compared to the drug-free control well for other drugs. In addition to a more user-friendly method, the newer versions of M27 provided both QC and reference MIC ranges with break points being provided for *Candida* species. To date, interpretive guidelines have only been established for 5-fluorocytosine; some azoles including fluconazole, itraconazole, and voriconazole; and the currently available candins anidulafungin, caspofungin, and micafungin. Categories for 5-fluorocytosine include susceptible (S), intermediate (I), and resistant (R) while those for the azoles include susceptible (S), susceptible-dose-dependent (SDD), and resistant (R). The susceptible-dose-dependent category relates to yeast testing only and is not interchangeable with the intermediate category associated with bacterial and 5-fluorocytosine break points. This category is in recognition that yeast susceptibility is dependent on achieving maximum blood levels. By maintaining blood levels with higher doses of the antifungal, an isolate with an SDD endpoint may be successfully treated with a given azole [1]. The candins are categorized only as susceptible or nonsusceptible. The term nonsusceptible is reserved for this group because, to date, insufficient data exists to create the resistance category.

Procedures for mould testing were released in 2002 as document M38-A [7]. Parameters were similar to those for yeast microtiter testing with the exception of the inoculum size which was increased approximately one log. Endpoint determination differed slightly, with zero visible growth considered the endpoint for amphotericin B, itraconazole, posaconazole, and voriconazole. The endpoints for the remaining azoles and 5-fluorocytosine continued to occur at the lowest concentration with a 50% reduction in growth as compared to the drug-free control well.

Realizing that the candins could not be read in the same manner as existing drugs, a new criterion for endpoint determination was required. The minimum effective concentration (MEC) was described to assist with this group [8–10]. The MEC is a more difficult endpoint to describe and only applies to mould testing. It is a result of the aberrant growth noted when many mould species come in contact with the candins. This aberrant growth is noted in the test wells but typically continues through the highest concentrations. The MEC is the lowest concentration where aberrant growth is first noted.

The time- and labor-intensive methods for antifungal susceptibility testing were difficult for many routine laboratories to incorporate into their workflow. As a result, the committee reviewed the feasibility of adopting a disk diffusion method for yeast testing. The resulting document, M44-A [4], released in 2004, provided an alternative for categorizing yeast as susceptible, susceptible-dose-dependent, or resistant without testing for MICs. This methodology utilizes Mueller-Hinton agar which is already a staple in most routine microbiology settings. Results are available in 24–48 h, and categorical placement of isolates falls very close to those determined by the MIC provided in broth methods.

2.3 Yeast Testing

Following the recruitment of several laboratories from across the United States, a preliminary standard was introduced 7 years following the initial committee report. This standard, M27-P [6], provided guidelines and stipulated the parameters that are still in effect. The most current method for antifungal susceptibility testing of yeast fungi is outlined in CLSI document M27-A3 [1]. It is important to note that only *Candida* spp. and *Cryptococcus neoformans* have been evaluated. Despite this fact, other species are frequently tested using these parameters. Current parameters for yeast testing include RPMI-1640 as the test medium; an inoculum size of 0.5–2.5×10^3 CFU/ml prepared spectrophotometrically; incubation at 24, 48, or 72 h depending on species and/or drug; and endpoint determinations of optically clear for amphotericin B or 50% reduction in turbidity for the other drugs in the microtiter system. Endpoints are slightly different when performing testing via the macrobroth method. Endpoints for AMB remain at optically clear, but endpoints for the remaining drugs are considered at the lowest concentration that results in an 80% reduction in turbidity as compared to the drug-free control tube.

Break points for the yeast are placed into one of five categories. These categories include susceptible, intermediate, susceptible-dose-dependent, resistant, and nonsusceptible. Isolates with MICs in the susceptible range indicate that the isolate is inhibited by a concentration of antifungal that is typically achieved in patients being treated by a standard dose. Currently, 5-flurocytosine is the only antifungal where the intermediate category is applicable. Isolates with MICs in this category are susceptible at a concentration that may be achieved in patients being given a standard dose but that are less likely to respond to therapy than an isolate that is considered susceptible. Susceptible-dose-dependent is a category unique to antifungal testing. This category indicates that a given drug may be effective in patients that can be treated with higher than normal doses and where maximum blood or tissue concentrations can be achieved. Resistant indicates that the isolate is not inhibited by concentrations of a drug that are typically achievable in patients. With the new class of antifungals, the candins, only two categories are being considered. These categories include susceptible and nonsusceptible. Nonsusceptible is used to categorize isolates that do not fall within the susceptible range for a drug but where resistance has not yet been defined.

Endpoints may be read either visually or spectrophotometrically. Turbidity is graded from 0 to 4 with 0 indicating optically clear and 4 indicating no reduction in turbidity compared to the turbidity of the drug-free control well. Grading of the remaining numbers include 1 for slightly hazy wells, 2 when a prominent reduction in turbidity is noted (usually approximately 50%), and 3 for a slight reduction in turbidity. This grading scale may be difficult since a true 50% reduction in turbidity as determined spectrophotometrically typically is substantially more turbid than the eye recognizes when assessing a 50% reduction.

For amphotericin B, the endpoint is the lowest concentration that inhibits visual growth or an endpoint score of 0. The endpoint for the azoles, 5-fluorocytosine, and the candins is the concentration where there is a decrease in turbidity of approximately 50% or an endpoint score of 2. When read spectrophotometrically, the endpoint is determined mathematically where a score of 0 equates with an optical density typically 5% or less of the drug-free control well. The endpoint for 2 equates with an optical density between approximately 6% and 50%.

Originally, the MIC was determined at either 48 h for *Candida* sp. or 72 h for *Cryptococcus neoformans.* The standard now permits the reading of endpoints in as few as 24 h for amphotericin B, fluconazole, and the candins. The remaining drugs should be read only at 48 or 72 h in the case of *Cryptococcus neoformans*. While the recommendation for the candins permits only the 24-h time point for determining the MIC, both amphotericin B and fluconazole may be read at either 24 or 48 h for *Candida* species.

The M27-A3 document permits some deviations from the method that may be evaluated by laboratories for use in their setting. One important deviation includes the use of media other than RPMI-1640 for testing of some drugs or species. The most widely discussed modification centers around amphotericin B. Isolates tested in RPMI-1640 result in amphotericin B MICs that are very tightly clustered around 1.0 μg/ml. This does not permit the distinction between susceptible isolates and

potentially resistant ones. Antibiotic medium 3 provides a wider distribution of MIC values. Isolates with low MICs can easily be distinguished from those with much higher MICs. It is critical that clinicians determine which medium is being used when evaluating amphotericin B results. Concerns have been expressed regarding lot-to-lot variability with antibiotic medium 3. This, however, has not been observed by all testing facilities.

While this method was being developed in the United States, the European community began work on a standard method as well. The EUCAST (European Community Antifungal Susceptibility Testing) method, although similar, incorporated some revisions to the CLSI method to include the addition of a higher concentration of glucose to the RPMI-1640. This addition facilitates the rate of fungal growth allowing the MIC to be determined at 24 h as opposed to the original M27-A-mandated 48 h. Studies have shown that the two methods are equivalent despite these differences [11] and that a given set of isolates can expect the same categorical placement regardless of the method utilized.

Great interest in acquired resistance has surfaced regarding *Candida* species. Some feel that the widespread use of fluconazole has led to decreased susceptibility of *Candida* sp. to not only this agent but others within the azole class revealing cross-resistance. Although it is possible to find azole resistance in any given collection or clinical setting, such resistance is not as widespread as some may fear (Table 2.1). Species resistance can be assessed by the MIC_{50} and MIC_{90}. These two values represent the MIC at which 50% or 90% of the isolates tested fall at or below. It is not to be confused with the mean MIC nor the median MIC but rather is a reflection of the MICs obtained for a test set of isolates.

Despite the presence of resistance in the clinical setting, the MIC_{50} and MIC_{90} for most species fall within what is considered a susceptible range. Notable exceptions include *Candida glabrata* against the azoles, *Candida krusei* against fluconazole, and *Cryptococcus neoformans* against caspofungin. It is critical to note that caspofungin is not recommended for *C. neoformans* nor is fluconazole recommended for *Candida krusei.* Neither of these isolates should be tested against the respective drugs to which they are intrinsically resistant.

Early reports have described *Candida lusitaniae* resistance to AMB and have shown that this species possesses the possibility of developing resistance while the patient is on treatment. The first report involved a patient whose initial isolate was susceptible but whose subsequent isolates had developed AMB resistance [12]. Later reports have shown AMB resistance may exist even prior to exposure to AMB [13]. The expected rate of resistance for *C. lusitaniae* is 8–10% of any given stock collection.

When discussing utility of susceptibility testing and its correlation to patient outcome, it is best to reference the document by Rex and Pfaller [14]. Some assumptions may be made regarding the MIC and patient outcome. Rex and Pfaller propose the “90–60 Rule.” This rule states that infections caused by isolates that have MICs considered susceptible respond favorably to appropriate therapy approximately 90% of the time, whereas infections caused by isolates with MICs considered resistant respond favorably approximately 60% of the time.

Table 2.1 Susceptibility patterns for clinical yeast isolates collected from 2000 to 2009

		AMB	CAS	5FC	FLU	ITRA	VORI
C. albicans	**N tested**	**1,878**	**2,089**	**465**	**3,291**	**1,173**	**1,251**
	MIC range	0.06 to 2.0	≤0.03 to ≥16	≤0.125 to ≥64	≤0.125 to 64	≤0.015 to ≥8.0	≤0.015 to ≥8
	MIC_{50}	0.25	0.06	0.25	0.25	≤0.015	≤0.015
	MIC_{90}	0.25	0.125	2.0	1.0	0.5	1.0
C. glabrata	**N tested**	**1,338**	**1,866**	**348**	**2,326**	**961**	**1,169**
	MIC range	0.125 to ≥16	0.06 to ≥16	≤0.125 to 16	≤0.125 to ≥64	≤0.015 to ≥8.0	≤0.015 to ≥8
	MIC_{50}	0.25	0.125	≤0.125	8.0	1.0	0.5
	MIC_{90}	0.5	0.25	≤0.125	≥64	8.0	4.0
C. krusei	**N tested**	**219**	**285**	**47**	**213**	**114**	**196**
	MIC range	0.125 to 2.0	0.06 to 8.0	2.0 to 16	8.0 to >64	0.08 to 8.0	0.125 to 8.0
	MIC_{50}	0.25	0.25	8.0	32	0.25	0.5
	MIC_{90}	0.5	0.25	8.0	>64	0.5	1.0
C. neoformans	**N tested**	**313**	**72**	**186**	**444**	**166**	**223**
	MIC range	0.06 to 0.5	4 to >16	≤0.125 to >64	≤0.125 to 64	≤0.015 to 0.5	≤0.015 to 0.5
	MIC_{50}	0.25	16	8.0	2.0	0.06	0.06
	MIC_{90}	0.5	>16	8.0	8	0.25	0.25

MICs reported in μg/ml

AMB amphotericin B, *CAS* caspofungin, *5FC* 5-flurocytosine, *FLU* fluconazole, *ITRA* itraconazole, *VORI* voriconazole, *N tested* number tested, *MIC* minimum inhibitory concentration

Realizing that M27-A3 is very labor intensive and not easily incorporated into busy clinical settings, the CLSI introduced M44-A. This method is a disk diffusion method that is similar to the routine Kirby-Bauer method utilized globally for bacterial susceptibility testing. To date, only fluconazole and voriconazole have been standardized, but the committee has evaluated other antifungals against both yeast and moulds. This method utilizes the same Mueller-Hinton agar that is required for bacterial testing but stipulates the addition of methylene blue-glucose to assist with yeast growth and to enhance visualization of the zone diameters. While it is beneficial for yeast testing, it does not hold true when testing moulds and M51-P methods eliminate the addition of methylene blue preparations.

Methylene blue-glucose solution is added to the surface of the Mueller-Hinton agar and permitted to air dry prior to adding the yeast inoculum. Laboratories are likely to find that M44-A fits into their workflow more easily than M27-A3 and appreciate the added benefit of being much less costly. Much work has been done to provide QC limits to ensure this method has the same validity as the original M27-A3 [15].

Since approved methods have been developed, commercial products have been introduced to assist laboratories with AST. Systems that have been evaluated include the YeastOne system by Trek Diagnostics and the Etest by AB Biodisk. These methods are easy to incorporate into the routine laboratory and give equivalent results to M27-A3 [16–18]. In addition, automated methods are under development with the Vitek by bioMérieux having FDA approval for fluconazole. Prior to launching an AST program, institutions should consider the volume of testing they can expect. The method is inherently variable, and reproducibility can be a problem. Another problem is the availability of an individual to discuss interpretation of the testing with clinicians.

Clearly, antifungal susceptibility testing of yeast fungi has become routine in many settings, and physicians are relying on MIC data to assist with difficult clinical decisions. The release of testing systems by industry such as the Etest (AB Biodisk) and YeastOne panels (Trek Diagnostics, Sensititre) has made this testing a reality in routine microbiology laboratories. While many clinicians will order susceptibility testing, there continues to be much confusion regarding the use of the results. Sufficient data has been generated to suggest susceptibility trends for specific isolates against specific agents, but direct patient outcome-MIC correlation data is minimal. Despite the lack of correlation data, antifungal susceptibility testing continues to provide useful information to assist with patient care.

2.4 Mould Testing

A new mould method, CLSI M38-A2, was released in 2002. This method is nearly identical to M27-A3 with the exception of the inoculum size. The inoculum size is determined spectrophotometrically but to a higher desired final concentration of $0.4–5 \times 10^4$ CFU/ml. The guideline provides target percent transmission (%T) readings

based on conidial size that are listed by species. Isolates from the genera of *Aspergillus* spp., *Paecilomyces* spp., and *Sporothrix* spp. are measured at 80–82%T while species with larger conidia such as *Fusarium* spp., *Rhizopus* spp., and *Scedosporium* spp. are standardized to 68–70%T. Efforts are under way to determine the correct%T for most of the clinically significant fungi, but the list is not yet complete. When fungi not discussed in the M38-A2 are tested, laboratories must determine the correct %T through trial and error to achieve the desired final concentration.

From several years of the use of M27 documents, it was recognized that the scientific community preferred the microtiter method to a macrobroth one. As a result, the macrobroth method is not discussed in M38-A2. This poses a problem when testing the endemic fungi such as *Histoplasma capsulatum, Blastomyces dermatitidis,* or *Coccidioides immitis*. When necessary, mould testing may be conducted by the macrobroth method as early studies have shown that the two methods are equivalent. Other fungi that may benefit from testing by the macrobroth method are those fungi that grow very slowly. It is difficult to hold microtiter tests longer than 72 h due to dehydration. Many of the less frequently encountered fungi may require as long as 120–144 h before growth is detected in the drug-free growth control well. For this reason, isolates that are known to be slow growers should be tested via the macrobroth method.

Endpoint determination is also much more difficult with moulds than with the yeast fungi. While a reduction in turbidity is typically easy to visualize with yeast fungi, it is not so easily visualized when moulds are tested. Due to the unique growth patterns of the mould fungi, one looks for a decrease in volume of growth rather than a reduction in turbidity as for the yeasts. *Aspergillus* spp., for example, growth is seen as a cottony clump in the broth. To determine an endpoint, the reader must assess the amount of growth for each concentration and call the endpoint at that concentration that has at least 50% smaller volume of growth for antifungals not read at 100% inhibition. Many individuals are not comfortable with this subjective endpoint determination and prefer to refer mould testing to reference centers.

Reading the MIC endpoint for moulds differs from the criteria established for the yeast fungi. Amphotericin B, itraconazole, posaconazole, and voriconazole endpoints are all determined at the lowest concentration that prevents discernable growth or in other words, the first clear well. Fluconazole and 5-fluorocytosine are determined at the lowest concentration that correlates with a 50% reduction in growth as the MIC. The candins do not provide a MIC but rather a MEC, or minimum effective concentration. The candins attack the growing tips of the hyphae resulting in aberrant, stubby growth of the hyphae. This aberrant growth is easily visualized as the hyphae cluster within the well in clumps. The MEC is the lowest concentration where the growth within the well is visually clumped. Microscopic examination will display obviously distorted hyphae.

Work has not been completed that permits categorizing moulds as susceptible or not. General guidelines have been established to assist with analyzing mould data. Based on large amounts of data [19], isolates are considered susceptible to amphotericin B, itraconazole, posaconazole, voriconazole, and caspofungin when the

MIC/MEC is ≤1.0 μg/ml, intermediate with MIC/MEC is 2.0 μg/ml, and resistant when the MIC/MEC is ≥4.0 μg/ml. It is likely that the other candins would fit into these ranges as well.

2.5 Conclusion

Antifungal susceptibility testing has indeed come of age. Physicians have discovered its utility and accepted its limitations when seeking assistance with tough clinical cases. While moulds are typically not tested in routine settings, yeast fungi are more frequently incorporated into clinical laboratories. As more drugs reach the market, the CLSI will be challenged to expand existing documents, especially when new classes of drugs are introduced. In the interim, the CLSI continues to monitor medical mycology to ensure appropriate methods are available for clinical testing.

References

1. CLSI (2008a) Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition; CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne
2. CLSI (2008b) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne
3. CLSI (2009) Method for antifungal disk diffusion susceptibility testing of filamentous fungi; proposed guideline. CLSI document M51-P. Clinical and Laboratory Standards Institute, Wayne
4. NCCLS (2004) Reference method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline. NCCLS document M44-A. National Committee for Clinical Laboratory Standards, Wayne
5. NCCLS (1985) Antifungal susceptibility testing; committee report. NCCLS document M20-CR. NCCLS, Villanova
6. NCCLS (1992) Reference method for broth dilution antifungal susceptibility testing of yeasts; proposed standard NCCLS document M27-P. National Committee for Clinical Laboratory Standards, Wayne
7. NCCLS (2002) Reference method for broth dilution antifungal susceptibility testing of conidial-forming filamentous fungi. Approved standard NCCLS M38-A. National Committee for Clinical Laboratory Standards, Wayne
8. Odds FC, Motyl M, Andrade R et al (2004) Interlaboratory comparison of results of susceptibility testing with caspofungin against *Candida* and *Aspergillus* species. J Clin Microbiol 42:3475–3482
9. Espinel-Ingroff A (2003) Evaluation of broth microdilution testing parameters and agar diffusion Etest procedure for testing susceptibilities of *Aspergillus* spp. to caspofungin acetate (MK-0991). J Clin Microbiol 41:403–409
10. Espinel-Ingroff A, Fothergill A, Ghannoum MA, Manavathu E, Ostrosky-Zeichner L, Pfaller M, Rinaldi M, Schell W, Walsh T (2007) Quality control and reference guidelines for CLSI broth micro-dilution method (M38-A document) for susceptibility testing of anidulafungin against moulds. J Clin Microbiol 45(7):2180–2182

11. Espinel-Ingroff A, Barchiesi F, Cuenca-Estrella M, Pfaller MA, Rinaldi M, Rodriguez-Tudela JL et al (2005) International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibilities of *Candida* spp. to fluconazole, itraconazole, posaconazole, and voriconazole. J Clin Microbiol 43(8):3884–3889
12. Pappagianis D, Collins MS, Hector R, Remington J (1979) Development of resistance to amphotericin B in *Candida lusitaniae* infecting a human. Antimicrob Agents Chemother 16:123–126
13. Merz WG (1984) Candida lusitaniae: frequency of recovery, colonization, infection, and amphotericin B resistance. J Clin Microbiol 20:1194–1195
14. Rex JH, Pfaller MA (2002) Has antifungal susceptibility testing come of age? Clin Infect Dis 35(8):982–989
15. Barry A, Bille J, Brown S, Ellis D, Meis J, Pfaller M et al (2003) Quality control limits from fluconazole disk susceptibility tests on Mueller-Hinton agar with glucose and methylene blue. J Clin Microbiol 41(7):3410–3412
16. Espinel-Ingroff A, Pfaller M, Messer SA, Knapp CC, Holliday N, Killian SB (2004) Multicenter comparison of the Sensititre YeastOne colorimetric antifungal panel with the NCCLS M27-A2 reference methods for testing new antifungal agents against clinical isolates of *Candida* spp. J Clin Microbiol 42(2):718–721
17. Maxwell MJ, Messer SA, Hollis RJ, Boyken L, Tendolkar S, Diekema DJ et al (2003) Evaluation of Etest method for determining fluconazole and voriconazole MICs for 279 clinical isolates of *Candida* species infrequently isolated from blood. J Clin Microbiol 41(3):1087–1090
18. Maxwell MJ, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA (2003) Evaluation of Etest method for determining voriconazole and amphotericin B MICs for 162 clinical isolates of *Cryptococcus neoformans*. J Clin Microbiol 41(1):97–99
19. Espinel-Ingroff A, Arthington-Skaggs B, Iqbal N, Ellis D, Pfaller MA, Messer S, Rinaldi M, Fothergill A, Gibbs D, Wang A (2007) Multicenter evaluation of a new disk agar diffusion method for susceptibility testing of filamentous fungi with voriconazole, posaconazole, itraconazole, amphotericin B, and caspofungin. J Clin Microbiol 45(6):1811–1820

http://www.springer.com/978-1-58829-847-8

Interactions of Yeasts, Moulds, and Antifungal Agents
How to Detect Resistance
Hall, G.S. (Ed.)
2012, XIV, 170 p., Hardcover
ISBN: 978-1-58829-847-8
A product of Humana Press