JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1996, p. 816–817 Vol. 34, No. 4
0095-1137/96/$04.00+0

Quality Control Guidelines for National Committee for Clinical Laboratory Standards-Recommended Broth Macrodilution Testing of Ketoconazole and Itraconazole

JOHN H. REX,[1]* MICHAEL A. PFALLER,[2] MICHAEL LANCASTER,[3] FRANK C. ODDS,[4] ANNE BOLMSTRÖM,[5] AND MICHAEL G. RINALDI[6]

Center for Infectious Diseases, Department of Internal Medicine, University of Texas Medical School, Houston, Texas 77030[1]; Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa 52242[2]; Alamar Biosciences, Inc., Sacramento, California 95834-1219[3]; Janssen Research Foundation, B-2340 Beerse, Belgium[4]; AB Biodisk, S-171 36, Solna, Sweden[5]; and Laboratory Service, University of Texas Health Science Center, Audie L. Murphy Memorial Veterans Hospital, San Antonio, Texas 78284-7750[6]

Received 18 September 1995/Returned for modification 17 November 1995/Accepted 3 January 1996

Ketoconazole and itraconazole were tested in a multilaboratory study to establish quality control (QC) guidelines for yeast antifungal susceptibility testing. Two isolates that had been previously identified as QC isolates for amphotericin B, fluconazole, and flucytosine (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were tested in accordance with the National Committee for Clinical Laboratory Standards M27-P guidelines. Each isolate was tested 20 times with the two antifungal agents in the five laboratories by using a lot of RPMI 1640 unique to each laboratory as well as a lot common to all five laboratories, thus generating 200 MICs per drug per organism. Overall, 96 to 99% of the MICs for each drug fell within the desired 3-$\log_2$ dilution range (mode ± 1 $\log_2$ dilution). By using these data, 3-$\log_2$ dilution QC ranges encompassing 98% of the observed MICs for three of the organism-drug combinations and 94% of the observed MICs for the fourth combination were established. These QC ranges are 0.064 to 0.25 μg/ml for both ketoconazole and itraconazole against *C. parapsilosis* ATCC 22019 and 0.125 to 0.5 μg/ml for both ketoconazole and itraconazole against *C. krusei* ATCC 6258.

The National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antifungal Susceptibility Testing has over the past decade coordinated development of M27-P, a reproducible method for broth dilution susceptibility testing of yeasts (2–4, 8). As part of the development of this method, the subcommittee has recently sought to identify suitable quality control (QC) isolates and MIC ranges. In an initial study, 10 candidate QC isolates were screened against amphotericin B, fluconazole, and flucytosine (6). Subsequently, two QC isolates and ranges for these three drugs were qualified by the NCCLS M23-A method (5, 7). The goal of the present study was to determine if these two QC isolates also exhibited sufficiently reproducible results for ketoconazole and itraconazole to permit their qualification as QC isolates for these two drugs.

MATERIALS AND METHODS

Antifungal agents. Ketoconazole and itraconazole were supplied by the Janssen Research Foundation (Beerse, Belgium). As specified by the M27-P method (4), stock solutions of each antifungal agent in dimethyl sulfoxide were prepared by Alamar Biosciences (Sacramento, Calif.) and frozen in small volumes at −20°C or lower until use. A twofold dilution range from 0.016 to 16 μg/ml was used for both drugs.

Yeast isolates. Two previously identified QC strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258) were used (7).

Study design and susceptibility testing methods. The study followed the NC CLS M23-A method for determination of QC ranges (5). The lots of RPMI 1640 used were lots 3040 and 3002 from American Biorganics, Inc. (Niagara Falls, N.Y.), lot ABA0195B from HyClone Laboratories, Inc. (Logan, Utah), lot 2N3982 from JRH Biosciences (Lenexa, Kans.), lot 951221120 from Irvine Scientific (Santa Ana, Calif.), and lot 13H46471 from Sigma Chemical Co. (St. Louis, Mo.). The media were prepared by following the manufacturers' recommendations and included glutamine but not bicarbonate and were buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS). All medium lots were prepared by Alamar Biosciences and supplied to the study laboratories in prepackaged containers.

Five laboratories participated in the study. Each laboratory performed broth macrodilution susceptibility testing according to the NCCLS M27-P guidelines (4) by using both a unique lot of RPMI 1640 and a lot that was common to all five laboratories (the common lot was lot 3040 from American Biorganics). Each laboratory tested each organism-drug combination 20 times using that laboratory's unique lot of RPMI 1640 as well as the common lot, generating a total of 200 MICs per drug per organism. Previously recommended statistical techniques were used to define the QC ranges (1). In brief, the proposed range should include the modal MIC ± 1 $\log_2$ dilution (1, 5) as well as ≥95% of the observed MICs.

TABLE 1. Distribution of broth macrodilution MICs of ketoconazole for two QC isolates in five laboratories[a]

Organism and type of RPMI 1640	No. of occurrences of MIC (μg/ml) of:				
	0.032	0.064	0.125	0.25	0.5
C. parapsilosis ATCC 22019					
Unique lot	1	33	63	3	
Common lot	4	31	62	3	
Total	5	[64	125	6]	
C. krusei ATCC 6258					
Unique lot			18	45	37
Common lot			3	59	38
Total			[21	104	75]

[a] Brackets enclose the proposed QC ranges. Blank cells indicate no occurrences.

* Corresponding author. Mailing address: Center for Infectious Diseases, Department of Internal Medicine, University of Texas Medical School, 6431 Fannin, 1728 JFB, Houston, TX 77030. Phone: (713) 792-4929. Fax: (713) 792-4937. Electronic mail address (Internet): jrex@heart.med.uth.tmc.edu.

TABLE 2. Distribution of broth macrodilution MICs of itraconazole for two QC isolates in five laboratories[a]

Organism and type of RPMI 1640	No. of occurrences of MIC (μg/ml) of:				
	0.032	0.064	0.125	0.25	0.5
C. parapsilosis ATCC 22019					
Unique lot		15	73	12	
Common lot	2	16	64	18	
Total	2	[31	137	30]	
C. krusei ATCC 6258					
Unique lot		12	48	40	
Common lot		1	19	80	
Total		[13	67	120	0]

[a] Brackets enclose the proposed QC ranges. Blank cells indicate no occurrences.

TABLE 3. Recommended MIC limits of ketoconazole and itraconazole for two QC strains

Organism	Antifungal agent	MIC range (μg/ml)	% of MICs within range
C. parapsilosis ATCC 22019	Ketoconazole	0.064–0.25	98
	Itraconazole	0.064–0.25	99
C. krusei ATCC 6258	Ketoconazole	0.125–0.5	100
	Itraconazole	0.125–0.5	94

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the MICs obtained by the five laboratories for ketoconazole and itraconazole for the two QC isolates. Overall, 98% of the ketoconazole MICs and 96% of the itraconazole MICs fell within a 3-$\log_2$ dilution range (mode $\pm$ 1 $\log_2$ dilution). QC ranges for each organism-drug combination were then assigned as the range containing the modal MIC $\pm$ 1 $\log_2$ dilution (Table 3). The range for the modal MIC $\pm$ 1 $\log_2$ dilution corresponded precisely with the MIC range that contained $\geq$95% of the observed MICs for three of the four organism-drug combinations. For the fourth combination, itraconazole and *C. krusei* ATCC 6258, the range for the mode $\pm$ 1 $\log_2$ dilution contained only 94% of the observed MICs. However, the results for this organism-drug combination were skewed by the results for one laboratory, where the unique lot of RPMI 1640 gave a value of 0.064 μg/ml 10 times and a value of 0.125 μg/ml 10 times. If these results are ignored, the proposed three-dilution QC range contains 98% of the remaining observed MICs. This proposed QC range is further somewhat problematic in that $\geq$94% of the observed values were actually distributed over a narrower two-dilution range. While a more stringent two-dilution QC range could be proposed, such a proposal would not accommodate the statistical outliers that could be expected during repeated testing and may be unrealistic (1). It thus seems most appropriate to follow the recommendations and precedent of Barry et al. (1) and propose a three-dilution range.

These results demonstrate that the QC strains previously selected for amphotericin B, fluconazole, and flucytosine also yield highly reproducible results when tested against ketoconazole and itraconazole by the M27-P method. The reproducibility of results with ketoconazole and itraconazole is comparable to that of results with amphotericin B, fluconazole, and flucytosine (7), as well as to results with antibacterial-agent–bacterium combinations (1). Thus, these two isolates and their corresponding QC ranges (Table 3) can now be used as QC performance guidelines for the NCCLS M27-P method.

In both this and prior studies (6, 7), we made no attempt to qualify strains that appeared resistant to any given antifungal agent, nor did we use isolates of any particular species. Rather, our goal was to identify isolates that gave results with excellent reproducibility. The NCCLS M23-A guidelines (5) were used to identify isolates for which reliable MICs that were not bounded by the extreme values of the standard dilution series could be obtained. We have now identified a pair of isolates that fit these requirements for all five commonly used systemic antifungal agents, and these isolates can be used both in the training of laboratory personnel and in the development of alternative methods equivalent to the NCCLS M27-P reference method. Use of these isolates in future studies will facilitate inter- and intralaboratory reproducibility of results and is a necessary step in the ongoing process of developing interpretive breakpoints for these antifungal agents.

ACKNOWLEDGMENTS

This study was supported by a grant from the Janssen Research Foundation and by Alamar Biosciences, Inc.

REFERENCES

1. **Barry, A. L., P. C. Fuchs, R. N. Jones, and the Collaborative Antimicrobial Susceptibility Testing Group.** 1989. Statistical criteria for selecting quality control limits for broth microdilution susceptibility tests for 39 different antimicrobial agents. Diagn. Microbiol. Infect. Dis. **12:**413–420.
2. **Espinel-Ingroff, A., C. W. Kish, Jr., T. M. Kerkering, R. A. Fromtling, K. Bartizal, J. N. Galgiani, K. Villareal, M. A. Pfaller, T. Gerarden, M. G. Rinaldi, and A. Fothergill.** 1992. Collaborative comparison of broth macrodilution and microdilution antifungal susceptibility tests. J. Clin. Microbiol. **30:**3138–3145.
3. **Fromtling, R. A., J. N. Galgiani, M. A. Pfaller, A. Espinel-Ingroff, K. F. Bartizal, M. S. Bartlett, B. A. Body, C. Frey, G. Hall, G. D. Roberts, F. B. Nolte, F. C. Odds, M. G. Rinaldi, A. M. Sugar, and K. Villareal.** 1993. Multicenter evaluation of a broth macrodilution antifungal susceptibility test for yeasts. Antimicrob. Agents Chemother. **37:**39–45.
4. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
5. **National Committee for Clinical Laboratory Standards.** 1994. Development of *in vitro* susceptibility testing criteria and quality control parameters. Approved guideline M23-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
6. **Pfaller, M. A., M. Bale, B. Buschelman, M. Lancaster, A. Espinel-Ingroff, J. H. Rex, and M. G. Rinaldi.** 1994. Selection of candidate quality control isolates and tentative quality control ranges for in vitro susceptibility testing of yeast isolates by National Committee for Clinical Laboratory Standards proposed standard methods. J. Clin. Microbiol. **32:**1650–1653.
7. **Pfaller, M. A., M. Bale, B. Buschelman, M. Lancaster, A. Espinel-Ingroff, J. H. Rex, M. G. Rinaldi, C. R. Cooper, and M. R. McGinnis.** 1995. Quality control guidelines for National Committee for Clinical Laboratory Standards recommended broth macrodilution testing of amphotericin B, fluconazole, and flucytosine. J. Clin. Microbiol. **33:**1104–1107.
8. **Rex, J. H., M. A. Pfaller, M. G. Rinaldi, A. Polak, and J. N. Galgiani.** 1993. Antifungal susceptibility testing. Clin. Microbiol. Rev. **6:**367–381.