JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1997, p. 270–272 Vol. 35, No. 1
0095-1137/97/$04.00+0

Lot-to-Lot Variability of Antibiotic Medium 3 Used for Testing Susceptibility of *Candida* Isolates to Amphotericin B

MARIO LOZANO-CHIU,[1]* PAGE W. NELSON,[1] MICHAEL LANCASTER,[2] MICHAEL A. PFALLER,[3] AND JOHN H. REX[1]

Center for Infectious Diseases, Department of Internal Medicine, University of Texas Medical School, Houston, Texas 77030[1]; SensiTitre, Westlake, Ohio 44145[2]; and Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa 52242[3]

Received 1 July 1996/Returned for modification 13 September 1996/Accepted 11 October 1996

We have previously shown that use of antibiotic medium 3 in the microdilution variant of the M27-T test method permits detection of amphotericin B-resistant *Candida* isolates. As this medium is not standardized and our initial work used only a single lot of antibiotic medium 3, we studied the lot-to-lot variability of three commercial lots of antibiotic medium 3 obtained from two sources. The MICs obtained with the new lots were lower than those of with the original lot, but the new lots still consistently separated putatively resistant and putatively susceptible isolates and this permits proposal of possible breakpoints for this assay system.

While intensive work over more than 10 years has led to development of M27-T, a standardized and highly reproducible method for testing of yeasts (2), we have recently shown that this method has only a limited ability to identify *Candida* isolates that are resistant to amphotericin B (6, 11). If, however, the M27-T method is altered by substituting antibiotic medium 3 (also known as Penassay broth) for the RPMI 1640 medium recommended by M27-T, then the assay system provides reliable discrimination between amphotericin B-resistant and -susceptible *Candida* isolates (6, 11). Unfortunately, antibiotic medium 3 is a nonstandardized medium containing yeast extract, beef extract, and gelatin (or peptone) (10), and substantial lot-to-lot variation is thus possible. In this study, we examined this problem by testing multiple lots of antibiotic medium 3 in our adaptation of the M27-T method.

(This work was presented in part as abstract F84 at the 96th General Meeting of the American Society for Microbiology, New Orleans, La., 19–23 May 1996.)

A collection of previously described putatively amphotericin B-susceptible and -resistant isolates was used (Table 1). A previously described quality control strain (*Candida parapsilosis* ATCC 22019) was also tested (3, 4), as were 64 previously described *Candida* blood isolates (43 isolates of *C. albicans*, 6 of *C. glabrata*, 1 of *C. krusei*, 8 of *C. parapsilosis*, and 6 of *C. tropicalis*) obtained from patients enrolled in a candidemia therapy trial (5–7). Of these 64 bloodstream isolates, 32 were from 32 patients treated with amphotericin B and for whom the outcome of this therapy is known. The organisms were kept at −70°C and were passed at least twice on Sabouraud dextrose agar at 35°C prior to being tested.

Three recently manufactured lots of antibiotic medium 3 were obtained: lot JD4ZSG from BBL Microbiology Systems (Cockeysville, Md.) and lots 36557JE and 47410JB from Difco Laboratories (Detroit, Mich.). These are referred to here as lots 1, 2, and 3, respectively. In all cases, the medium lots were filter sterilized after reconstitution and supplemented with glucose to achieve a final glucose concentration of 2% (2 g/100 ml). All lots were buffered by adding 1 g of dipotassium monophosphate per liter and 1 g of monopotassium monophosphate per liter and adjusting the pH to 7.0. Preparation of the yeast inoculum and drugs followed the National Committee for Clinical Laboratory Standards M27-T method (2), with the exception of three details. First, the test medium was antibiotic medium 3 prepared as described above. Second, as previously described (6), we employed the microdilution adaptation that is now incorporated in M27-T as an alternative method. We determined MICs by measuring optical density at 530 nm with an EL-310 plate reader (BIO-TEK, Burlington, Vt.) after both 24 and 48 h of incubation at 35°C. The plates were agitated prior to reading, and the MIC was the lowest concentration of amphotericin B which completely inhibited fungal growth.

The MICs of the three lots of antibiotic medium 3 obtained with the test isolates at 24 h are shown in Table 2. With all three medium lots, the MICs for the susceptible isolates were ≤0.125 μg/ml, while the MIC range for the resistant isolates was 0.25 to 16 μg/ml. The results for the individual isolates were very consistent within and between the three medium lots, with all of the MICs being within 1 dilution of the modal MIC for all isolates except Y537: CL524, range of ≤0.0625 to 0.125 μg/ml and mode of ≤0.0625 μg/ml; ATCC 22019, range of ≤0.0625 to 0.125 μg/ml and mode of ≤0.0625 μg/ml; 5W31, range of 0.5 to 1 μg/ml and mode of 0.5 μg/ml; CL2819, range of 0.5 to 1 μg/ml and mode of 0.5 μg/ml; Y533, range of 0.25 to 0.5 μg/ml and mode of 0.5 μg/ml; Y534, range of 0.25 to 0.5 μg/ml and mode of 0.25 μg/ml; MY1012, range of 4 to 16 μg/ml and mode of 8 μg/ml. For isolate Y537, a range of 0.5 to 2 μg/ml and a mode of 0.5 μg/ml were obtained. As was noted in our previous study (6), this good-to-excellent discrimination between susceptible and resistant isolates was lost if the MIC was determined after 48 h of incubation (data not shown). As the three commercially obtained lots of antibiotic medium 3 were the only lots available for purchase at the time of this study, we also prepared three additional lots of antibiotic medium 3 by combining three different lots of yeast extract (1.5 g/liter) from different vendors (lots D5DAZA and G6DDDC from BBL and lot 791758 from Difco) with beef extract (1.5 g/liter; lot 121H0062 from Sigma Chemical Co., St. Louis,

* Corresponding author. Mailing address: 6431 Fannin, 1728 JFB, Houston, TX 77030. Phone: (713) 792-4929. Fax: (713) 792-4937.

TABLE 1. *Candida* isolates used in this study

Isolate	Species	Putative status[a]
CL524	*C. parapsilosis*	Susceptible
ATCC 22019	*C. parapsilosis*	Susceptible
5W31	*C. lusitaniae*	Resistant
CL2819	*C. lusitaniae*	Resistant
Y533	*C. lusitaniae*	Resistant
Y534	*C. lusitaniae*	Resistant
Y537	*C. albicans*	Resistant
MY1012	*C. tropicalis*	Resistant

[a] Isolates 5W31, CL2819, and CL524 were proven susceptible or resistant to amphotericin B in an animal model of disseminated candidiasis (1). The other isolates are presumed to be susceptible or resistant based on results obtained by in vitro testing multiple times and under multiple conditions (6, 11).

Mo.), peptone (5 g/liter; lot 52584JB from Difco), and the required salts (10). These lots were sterilized, supplemented with glucose to achieve a final glucose concentration of 2% (2 g/100 ml), and buffered to pH 7.0 in the same manner as the commercial lots of antibiotic medium 3. Studies with these lots yielded similar but somewhat less consistent results (data not shown), thus further demonstrating the general reproducibility of susceptibility testing with this medium but also underscoring the importance of the quality control process employed during commercial manufacture of the complete medium.

These results do, however, differ from those of our previous study in that they suggest that MICs of ≥0.25 μg/ml should be classified as resistant, rather than the previously observed lower boundary of 1 μg/ml. This difference may be due to either interlot variability of antibiotic medium 3 or deterioration of antibiotic medium 3 over time: all of the results in our first study were obtained with a single, older lot of antibiotic medium 3 that is no longer available, whereas all of the lots used in this study were more recently manufactured. It is of note that inclusion of 0.25 μg/ml in the range for the resistant isolates is due to results obtained with only two isolates, Y533 and Y534. Neither of these isolates has been tested in an animal model, so their true susceptibility to amphotericin B remains uncertain. However, these isolates do consistently yield MICs that are higher than those obtained with most other isolates. This was especially true when testing was performed with the Etest method on antibiotic medium 3 agar (11): with this method, the MIC for 90% of the *Candida* isolates tested was 1 μg/ml, while the MICs for Y533 and Y534 were >32 and 4 μg/ml, respectively. The idea that isolates for which the MIC is 0.25 μg/ml are unusual is further supported by the results obtained for the 64 bloodstream isolates: the observed range was ≤0.0625 to 0.25 μg/ml, and the modal MIC was 0.0625 μg/ml. Although the observed range of MICs included 0.25 μg/ml, this was due to a single isolate of *C. glabrata* and the 63 remaining isolates had MICs of ≤0.125 μg/ml. It is interesting that when the MIC was compared with the outcome for the 32 isolates from patients treated with amphotericin B, the same MIC range (0.0625 to 0.125 μg/ml) was seen for the 27 isolates from successfully treated patients and the 5 isolates from patients who either failed therapy or relapsed after the end of therapy (the isolate of *C. glabrata* with an MIC of 0.25 μg/ml was from a patient treated with fluconazole). This frequency of failure with apparently susceptible isolates is typical of MIC-outcome correlations in this and related settings (7–9) and presumably related to interactions among host defenses, underlying disease, and the infecting organism. Based on these results, we consider isolates with MICs of ≤0.125 μg/ml to be susceptible and those with MICs of ≥0.5 μg/ml to be resistant. The implication of an MIC of 0.25 μg/ml is uncertain, since the behavior of the relevant isolates has not been validated in an animal model. Nonetheless, the data do indicate that isolates for which the MIC is 0.25 μg/ml are uncommon and, at the very least, should be judged to have a somewhat lessened susceptibility to amphotericin B.

TABLE 2. Microdilution M27-T MICs obtained at 24 h

Lot and isolates[a]	MIC range (μg/ml)	Modal MIC (μg/ml)
1		
S	≤0.0625	0.0625
R	0.25–16	1
2		
S	≤0.0625–0.125	0.0625
R	0.25–16	0.5
3		
S	≤0.0625–0.125	0.0625
R	0.25–16	0.5

[a] S, susceptible isolates CL524 and ATCC 22019; R, resistant isolates 5W31, CL2819, Y533, Y534, Y537, and MY1012. Each of the eight test isolates was tested 12 times with the indicated lot of antibiotic medium 3.

In conclusion, we observed minimal lot-to-lot variability of antibiotic medium 3 in three recently obtained commercial lots of this medium when they were used to test the activity of amphotericin B against *Candida* isolates. By using all available data, possible interpretive breakpoints for this system can be suggested. The reproducibility of these results requires validation in a multilaboratory study.

This work was supported in part by a grant to M. Lozano-Chiu from the Sociedad Española de Quimioterapia of Spain.

REFERENCES

1. **Anaissie, E. J., N. C. Karyotakis, R. Hachem, M. C. Dignani, J. H. Rex, and V. Paetznick.** 1994. Correlation between in vitro and in vivo activity of antifungal agents against *Candida* species. J. Infect. Dis. **170:**384–389.
2. **National Committee for Clinical Laboratory Standards.** 1995. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard M27-T. National Committee for Clinical Laboratory Standards, Wayne, Pa.
3. **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.
4. **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.
5. **Rex, J. H., J. E. Bennett, A. M. Sugar, P. G. Pappas, C. M. Van der Horst, J. E. Edwards, R. G. Washburn, W. M. Scheld, A. W. Karchmer, A. P. Dine, M. J. Levenstein, C. D. Webb, the Candidemia Study Group, and the NIAID Mycoses Study Group.** 1994. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. N. Engl. J. Med. **331:**1325–1330.
6. **Rex, J. H., C. R. Cooper, Jr., W. G. Merz, J. N. Galgiani, and E. J. Anaissie.** 1995. Detection of amphotericin B-resistant *Candida* isolates in a broth-based system. Antimicrob. Agents Chemother. **39:**906–909.
7. **Rex, J. H., M. A. Pfaller, A. L. Barry, P. W. Nelson, and C. D. Webb for the NIAID Mycoses Study Group and the Candidemia Study Group.** 1995. Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B as treatment of nonneutropenic patients with candidemia. Antimicrob. Agents Chemother. **39:**40–44.
8. **Sanders, W. E., Jr., and C. C. Sanders.** 1982. Do in vitro antimicrobial susceptibility tests accurately predict therapeutic responsiveness in infected

patients? p. 325–340. *In* V. Lorian (ed.), Significance of medical microbiology in the care of patients, 2nd ed. The Williams & Wilkins Co., Baltimore, Md.
9. **Stratton, C. W., IV.** 1991. In vitro testing: correlations between bacterial susceptibility, body fluid levels and effectiveness of antibacterial therapy, p. 849–879. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore, Md.
10. **Vera, H. D., and D. A. Powder.** 1980. Culture media, p. 973. *In* E. H. Lennette,
11. **Wanger, A., K. Mills, P. W. Nelson, and J. H. Rex.** 1995. Comparison of Etest and National Committee for Clinical Laboratory Standards broth macrodilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant *Candida* isolates. Antimicrob. Agents Chemother. **39:**2520–2522.