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Correlation between In Vitro Susceptibility Determined by E Test and Response to Therapy with Amphotericin B: Results from a Multicenter Prospective Study of Candidemia

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Ninety-nine *Candida* bloodstream isolates underwent testing for susceptibility to amphotericin B by the E test, and the results were correlated with patients' responses to amphotericin B. The MICs for isolates that were associated with therapeutic failure were significantly higher than the MICs for those associated with therapeutic success. A MIC of ≥0.38 μg/ml identified isolates likely to be associated with therapeutic failure.

The proposal of interpretive breakpoint MICs of fluconazole and itraconazole that identify *Candida* isolates likely to fail to respond to therapy with these agents is a significant advance in antifungal susceptibility testing (14). In comparison, testing of *Candida* isolates for susceptibility to amphotericin B has lagged; standardized testing methods recommended by the National Committee for Clinical Laboratory Standards generate a narrow range of amphotericin B MICs, limiting the ability to identify isolates likely to cause therapeutic failure (8, 13). Recently, a simple testing method employing E-test strips identified two isolates known to be resistant to amphotericin B in animal models of candidiasis (15, 16), suggesting that the E test might be able to identify amphotericin B-resistant isolates recovered from humans. To address this possibility, we used the E test to determine amphotericin B MICs for 99 *Candida* bloodstream isolates recovered from patients enrolled in a multicenter prospective study of candidemia (8). MICs were correlated with the ability of amphotericin B to eliminate *Candida* organisms from patients' bloodstreams.

The *Candida* isolates were recovered from 99 candidemic patients who received amphotericin B as monotherapy (median dose, 0.6 mg/kg/day) and from whom all intravenous catheters were removed prior to enrollment. The isolates included were 52 of *Candida albicans*, 20 of *C. glabrata*, 14 of *C. tropicalis*, 9 of *C. parapsilosis*, 3 of *C. lusitaniae*, and 1 of *C. krusei*, each of which had previously been tested for amphotericin B susceptibility by the National Committee for Clinical Laboratory Standards broth macrodilution method (8, 9). For the E test, the *Candida* inoculum density was standardized to a 0.5 McFarland standard and 300 μl was dispensed onto the center of an 80-mm-diameter plate containing RPMI 1640 medium with L-glutamine supplemented with 2% glucose and buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS), with Bacto Agar added at a final concentration of 1.5 g/100 ml. MICs were interpreted as previously described (1). Thirty-three isolates were tested at least twice by the E test, and the MICs agreed within a fourfold dilution. Therapeutic failure was defined as failure of amphotericin B to clear *Candida* from the bloodstream on the basis of previously reported criteria (8).

The E test yielded a wide distribution of amphotericin B MICs (Table 1). The broth macrodilution method, on the other hand, had previously yielded a narrow distribution of MICs, ranging from 0.06 to 1 μg/ml (8). The amphotericin B MICs determined by the E test were higher for isolates associated with therapeutic failure than the MICs for those associated with therapeutic success. This finding was most pronounced at 48 h (geometric mean MICs of 0.22 and 0.05 μg/ml, respectively; $P = 0.001$), although the difference was also significant at 24 h (geometric mean MICs of 0.10 and 0.03 μg/ml, respectively; $P = 0.01$).

Based on the distribution of MICs at 24 and 48 h stratified by response to therapy, breakpoint MICs capable of identifying amphotericin B-resistant isolates could be proposed. At 24 h, 46% (15 of 33) of the isolates for which the MICs were ≥0.19 μg/ml were associated with therapeutic failure, versus only 17% (11 of 66) of those for which the MICs were <0.19 μg/ml ($P = 0.005$). The relative risk of therapeutic failure among patients infected with isolates for which the MICs at 24 h were ≥0.19 μg/ml was 2.7-fold (95% confidence interval, 1.5 to 12) greater than the risk of failure among patients infected with isolates for which the MICs were <0.19 μg/ml. At 48 h, 56% (14 of 25) of the isolates for which the MICs were ≥0.38 μg/ml were associated with therapeutic failure, versus only 16% (12 of 74) of the isolates for which the MICs were <0.38 μg/ml ($P = 0.0001$). The relative risk of therapeutic failure among patients infected with isolates for which the MICs at 48 h were ≥0.38 μg/ml was 3.5-fold (95% confidence interval, 2.2 to 20.4) greater than the risk of failure among patients infected with isolates for which the MICs were <0.38 μg/ml.

The E-test breakpoint MIC of ≥0.38 μg/ml had a sensitivity of 54% (14 of 26), a specificity of 85%, and a positive predictive value of 56%. The sensitivity and specificity of these breakpoint MICs are similar to those of the fluconazole breakpoint MIC recently proposed (14). Such values are impressive, given the inherent difficulty of detecting amphotericin B resistance among isolates recovered from the heterogeneous population of candidemic patients, whose therapeutic outcome depends upon myriad factors in addition to the susceptibility of the infecting organism to antifungal agents.

Although therapeutic failure of amphotericin B is well recognized in series of patients with candidemia, an association between such failure and amphotericin B resistance has been

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TABLE 1. Distribution of amphotericin B MICs determined by the E-test method

Amphotericin B MIC (μg/ml)	No. of isolates at 24, 48 h						
	C. albicans	*C. glabrata*	*C. tropicalis*	*C. parapsilosis*	*C. lusitaniae*	*C. krusei*	Total
≤0.125	42, 37	7, 5	7, 3	8, 4	2, 2	0, 0	66, 51
0.19	6, 5	3, 1	2, 2	0, 0	0, 0	0, 0	11, 8
0.25	0, 6	3, 3	4, 3	1, 3	0, 0	0, 0	8, 15
0.38	3, 3	1, 1	1, 4	0, 0	0, 0	1, 0	6, 8
0.5	0, 0	3, 7	0, 1	0, 1	0, 0	0, 1	3, 10
0.75	1, 1	0, 0	0, 1	0, 0	0, 0	0, 0	1, 2
≥1.5	0, 0	3, 3	0, 0	0, 1	1, 1	0, 0	4, 5

infrequently observed (2, 3, 5, 7, 10). The incidence of in vitro resistance among series of clinical isolates has generally been extremely low (13), leading some to suggest that susceptibility of *Candida* to amphotericin B is essentially universal (6) and that failure of therapy is primarily due to factors such as host immune status and underlying illness. Our results, however, argue that amphotericin B resistance does exist and contributes to at least some of the cases in which amphotericin B therapy fails. Our finding of a significant rate of amphotericin B resistance is consistent with an earlier report that found resistance among 7% of the *Candida* isolates recovered from oncology patients (2, 12). There are two possible reasons that these two studies identified a higher number of amphotericin B-resistant isolates than other studies. First, many of the isolates tested were recovered from patients who had received prior courses of amphotericin B or who were neutropenic, factors that likely predisposed to the emergence of resistance. Second, the agar-based methodologies of in vitro testing used in both studies might be more sensitive than broth-based methods of detecting amphotericin B resistance.

Earlier studies have investigated the E test as a method of testing *Candida* isolates for susceptibility to amphotericin B. Several have found E-test MICs to be comparable to those of the macrodilution method (4, 11). Our study corroborates these findings; when E-test MICs were rounded up to the next highest value that corresponded to a standard twofold dilution value used in the broth macrodilution method, the agreement within a fourfold dilution between these two methods was 96% (95 of 99).

In conclusion, we have demonstrated that the E test is a potentially useful method of identifying amphotericin B-resistant *Candida* isolates. If these results can be confirmed, the E test represents a significant advance in antifungal susceptibility testing. To facilitate the use of the E test in future studies of clinical yeast isolates and to permit comparison of results between such studies, the development of a standardized testing methodology is of the highest priority.

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