INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

(Official publication of Indian Association of Medical Microbiologists,
Published quarterly in January, April, July and October)
Indexed in Index Medicus/MEDLINE/PubMed, 'Elsevier Science - EMBASE', 'IndMED'

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Annual Subscription	Rs 2,000/-	US $ 150
Single Copy	Rs 600/-	US $ 75

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The journal is printed on acid free paper.

INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

(Publication of Indian Association of Medical Microbiologists)

ISSN 0255-0857 **Volume 25** **Number 4** **October-December, 2007**

CONTENTS

Evaluation of Glucose-Methylene-Blue-Mueller-Hinton Agar for E-Test Minimum Inhibitory Concentration Determination in *Candida* spp.

Dear Editor,

A rise in invasive fungal infections and their emerging resistance have necessitated the need for antifungal susceptibility testing (AFT) for clinical work-up.[1] The standardized broth micro-dilution (BMD) method is expensive, laborious and cumbersome for routine use in clinical microbiology laboratory.[2] Recently, a disc-diffusion method has been approved by CLSI using glucose-methylene-blue (GMB) Mueller-Hinton agar (MHA). Despite being easy and practical, this needs to be confirmed by BMD to exclude false resistance.[1]

Recent reports have documented comparable results between BMD (NCCLS-27-A)[3] and agar-based E-test.[4,5] The manufacturer-recommended media for E-test is glucose-supplemented RPMI agar (RPMI-G). The end point for azoles is poorly defined on this medium. Therefore, we undertook this study to determine whether GMB-MHA could be used in the E-test method.

A total of 31 blood stream isolates from candidaemia cases were selected. These were speciated using germ tube test, CHROM agar, cornmeal agar and tetrazolium reduction test (Himedia, Mumbai). Antifungal susceptibility of these isolates was performed by E-test and BMD for amphotericin-B and fluconazole. The E-strip (AB-Biodisk, Solna) minimum inhibitory concentration (MIC) was determined on RPMI-G (RPMI + 1.5% agar + 2% glucose) media and GMB-MHA (MHA + 2% glucose + 0.5 μg of methylene blue). For agar diffusion E-test, 0.5 McFarland standard inocula were applied to GMB-MHA and RPMI-G media with a cotton swab. The plates were allowed to dry for at least 15 min before the E-strip was applied to the surface. The MIC for the E-test was measured after 24 h, at transition point where growth abruptly decreased (reduction in colony, size, number and density: approximately 80% growth inhibition standards). BMD-MIC was performed using RPMI and 0.165 M morpholine propanesulphonic acid (Himedia, Mumbai). The interpretation was done spectrophotometrically after 24 and 48 h of incubation, as per NCCLS guidelines.[3] The optical density (OD) of the medium control well was subtracted from the ODs of all other wells and MIC concentration was computed mathematically. Briefly, the BMD-MIC of amphotericin B was determined as the lowest concentration with an OD corresponding to greater than or equal to 90% decrease in turbidity compared to that of growth control and the MICs of fluconazole, corresponding to a 50% decrease in turbidity.[3] The quality control was performed by testing *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) with each batch of clinical isolates. All the MIC experiments were repeated twice and mean was taken.

The isolates included in the study comprised of *C. tropicalis* (12), *C. parapsilosis* (8), *C. albicans* (8), *C. krusei* (2) and *C. glabrata* (1). Twenty-four (77.4%) of the isolates that were found to be susceptible by BMD were identified as susceptible by RPMI-G agar to amphotericin B and fluconazole. The similar figures for GMB-MHA was 24 (77.4%) and 25 (80.6%) for amphotericin B and fluconazole, respectively. Higher MIC levels (1-2 dilutions) were noted by E-strip method as compared to BMD method. Table shows the comparison of susceptibility by the E-test method on GMB-MHA and RPMI-G media.

Table: Comparison of susceptibility by the E-test method on RPMI-G and GMB-MHA

Candida spp.** (*n*)	Total no. of susceptible isolates			
	RPMI-G		GMB-MHA	
	Amphotericin B (μg/mL)*	Fluconazole (μg/mL)*	Amphotericin B (μg/mL)*	Fluconazole (μg/mL)*
C. tropicalis (12)	10	10	10	11
C. parapsilosis (8)	7	6	7	6
C. albicans (8)	7	8	7	8

*Interpretive criteria: amphotericin - ≤1 μg/mL (sensitive), >2 μg/mL (resistant); fluconazole - ≤8 μg/mL (sensitive), ≥64 μg/mL (resistant); **_C. krusei_ (2) and _C. glabrata_ (1) were resistant to amphotericin B and fluconazole on RPMI-G and GMB-MHA

Prior studies have shown that agar-based susceptibility testing (E-test and DD method) are more practical as compared to BMD due to their simplicity and reproducibility. Requirement of additional equipment (spectrophotometer) is also eliminated.[4] The results obtained by using both media were in acceptable concordance (>93%) with those obtained by MBD method. Similar findings were corroborated by previous studies.[4] Disparate readings were attributable to 'trailing growth phenomenon' in an isolate of *C. tropicalis*. This discrepancy was also observed in prior studies.[2,6]

A large number of recent studies have compared E-test or DD on different media.[1,2,6] But studies comparing E-test MIC on RPMIG and GMB-MHA could not be seen in the pertinent literature. The overall levels of agreement between E-strip MICs obtained by RPMIG and GMB-MHA at 24 h were comparable (97% agreement) for both amphotericin-B and fluconazole for resistant and susceptible isolates. However, a single disparity in an isolate of *C. tropicalis* in fluconazole susceptibility was observed, where RPMI-G and BMD gave a resistant and GMB-MHA depicted a susceptible MIC. This disparity could not be explained as repeat testing yielded the same result. Higher MIC levels (1-2 dilutions) were noted by E-strip method as compared to BMD method. These findings have been reported previously.[5]

It was observed in the current and prior studies that GMB-MHA has enhanced growth, simplified reading and minimal microcolonies around zone of inhibition.[1,4] Thus GMB-MHA appears to be a useful medium for E-test, as it compared well with BMD and RPMI-G (except a single *C. tropicalis* outlier despite repeat testing). The stock-solution of GMB can be refrigerated and added to the molten and cooled MHA which is made routinely. In a resource constraint and busy clinical laboratory, AFT by MIC method on GMB-MHA followed by confirmation by BMD of resistant strains is recommended to exclude false resistance. Further, meaningful large-scale studies and continued refinement of all susceptibility techniques are required for routine AFT.

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Received: 12-05-07
Accepted: 26-06-07