Comparison of the Suitability of Three Common Bacterial Media for Susceptibility Testing of Trimethoprim-Sulfamethoxazole

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The in vitro inhibitory activity of trimethoprim-sulfamethoxazole is inversely related to the amount of thymine and thymidine in the test medium; manufacturers must adequately control their media to avoid spurious antimicrobial susceptibility results. No differences were observed when commercial Mueller-Hinton broth and agar were compared with a semisynthetic broth medium by the use of microdilution and disk diffusion techniques.

The 20:1 combination of trimethoprim (TMP) and sulfamethoxazole (SMZ) is an effective antimicrobial compound used for treatment of a number of pathogenic bacteria (5). As with most drugs, susceptibility testing of the clinical isolate is indicated to predict the potential effectiveness of the drug in vivo.

Susceptibility testing of TMP-SMZ and other sulfa drugs must be performed in a medium devoid of thymine and thymidine (2, 6). If these constituents are present, they inhibit TMP-SMZ (and other sulfa) so that susceptible organisms appear resistant. Thus, to prevent an inaccurate susceptibility determination, commercial broth and agar media must be thymine and thymidine free.

This study was undertaken in an attempt to determine whether commercial commercially available media are satisfactory for testing TMP-SMZ. One commercial broth medium (Difco Laboratories, Detroit, Mich.) and two commercial agar media (Difco and BBL Microbiology Systems, Cockeysville, Md.) were compared with a semisynthetic thymine- and thymidine-free broth medium (Adams and Roe).

One hundred ten clinical isolates recovered in the microbiology laboratory at Cedars-Sinai Medical Center (Los Angeles, Calif.) were tested. These included 27 strains of Pseudomonas aeruginosa, 27 of Escherichia coli, 26 of Streptococcus faecalis, 3 of Pseudomonas maltophilia, 1 of Pseudomonas cepacia, 5 of Klebsiella pneumoniae, 4 of Klebsiella oxytoca, 5 of Proteus mirabilis, 3 of Acinetobacter anitratus, 6 of Enterobacter cloacae, 2 of Citrobacter freundii, and 1 of Citrobacter diversus.

Mueller-Hinton II agar plates and TMP-SMZ disks were purchased from BBL. Difco Mueller-Hinton agar plates and broth were prepared in-house from dehydrated base. The semisynthetic Adams and Roe broth and TMP and SMZ powders were kindly supplied by R. Cleeland (Hoffmann-La Roche Inc., Nutley, N.J.).

Agar disk diffusion tests were performed according to the recommendations of the National Committee for Clinical Laboratory Standards (3). The following interpretive standards were used: sensitive, \( \leq 16 \) mm; intermediate, 11 to 15 mm; resistant, \( \leq 10 \) mm. Slight growth (i.e., \( \geq 80\% \) inhibition) was disregarded in measuring zones of inhibition (3).

Microbroth dilution tests were performed according to the recommendations of the National Committee for Clinical Laboratory Standards (4). Twofold dilutions were prepared in microtiter trays, beginning with a dilution of 16/304 (TMP-SMZ) and ending with a dilution of 0.12/2.38 (TMP-SMZ). The following interpretive standards were used: susceptible, \( \leq 0.5/9.5 \) (TMP-SMZ); moderately susceptible, 1/19 to 2/38; conditionally susceptible, 4/76 to 8/152; resistant, >2/38.

Microorganisms were considered resistant if there was (i) definite turbidity in the well, (ii) a single cluster of growth \( \leq 2 \) mm, or (iii) more than one cluster of growth even if each was less than 2 mm in diameter (1).

The BBL Mueller-Hinton II and Difco Mueller-Hinton agar plates exhibited zones within 2 mm of each other, i.e., there were no differences in the susceptibility results for any of the bacteria tested. Minimum inhibitory concentrations of all the bacteria tested were within one dilution of each other with either the semisynthetic Adams and Roe broth or Difco Mueller-Hinton broth. Results of the agar disk diffusion susceptibility tests showed 100% agreement with all broth dilution tests.

Commercial Mueller-Hinton agar (BBL and Difco) and Mueller-Hinton broth (Difco) appear to be adequately controlled for thymine and thymidine content since susceptibility results with each of these media were comparable with those of the semisynthetic, thymine- and thymidine-free broth (Adams and Roe). Most commercially available microdilution systems, e.g., Sensititre (GIBCO Laboratories, Grand Island, N.Y.), Microscan (American Scientific Products, McGaw Park, Ill.), Sceptor (BBL), and Micromedia Systems (Micromedia-Systems, Inc., Potomac, Md.) use thymine- and thymidine-free Mueller-Hinton broth. These should provide accurate in vitro susceptibility data without the addition of supplements such as thymidine phosphorylase or lysed horse blood (which contains thymidine phosphorylase).

Broth susceptibility testing of TMP-SMZ is, in general, harder to interpret than agar disk diffusion testing, due to "trailing" of bacterial growth in the wells of the microtiter plates (Fig. 1). We found that it was important to measure the size of the bacterial button in the bottom of the wells since buttons <2 mm are not considered significant (Fig. 2), and false interpretations of resistance may occur if measurements are not taken (1).

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FIG. 1. Microdilution broth tests for S. faecalis ATCC 29212 (row A) and E. coli ATCC 25922 (row B). The minimum inhibitory concentration for S. faecalis is 0.12/2.38 \( \mu \)g of TMP-SMZ per ml (well B). The minimum inhibitory concentration for E. coli is 0.5/9.5 \( \mu \)g of TMP-SMZ per ml (well F). Well 10 is the growth control well.
FIG. 2. Enlargement of bacterial button shown in Fig. 1, row A, well 8 (S. faecalis ATCC 29212), showing a diameter less than 2 mm, i.e., the organism is inhibited by the lowest concentration of drug tested.

LITERATURE CITED