Inhibition of Bacterial Growth by the Nitrofurantoin Solvent Dimethylformamide

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For quantitative susceptibility tests, stock solutions of nitrofurantoin are often prepared in dimethylformamide. Since 1.0 or 2.0 ml of the solvent will inhibit microbial growth, currently recommended procedures for antimicrobial dilution tests should be modified by adding only 0.1 or 0.2 ml of stock drug solutions to the agar or broth medium.

For performing agar or broth dilution susceptibility tests, concentrated stock solutions of each antimicrobial must be prepared in an appropriate solvent. The nitrofurans are not very soluble in water and, thus, a solvent such as dimethylformamide (DMF) is often used for preparing the initial solution. In our experience, nitrofurantoin will dissolve in DMF at a concentration as great as 30 mg/ml, and serial dilutions of such concentrated solutions may be carried out in DMF until the solution contains less than 1 mg/ml, at which point further dilution may be performed in distilled water, diluting the drug and the DMF solvent with each dilution step.

According to the agar dilution procedures described by Ericsson and Sherris (1) and by Washington and Barry (2), stock solutions of antimicrobial should be diluted to contain 10 times the final concentration desired, and then one volume (2 ml) of the drug solution is added to nine volumes (18 ml) of the agar medium. For broth dilution tests, the stock solutions should contain twice the final concentration desired, with the final dilution step carried out in a broth medium. When testing nitrofurantoin, a final concentration of at least 512 μg/ml should be tested. Consequently, the stock solutions should contain 5,120 μg/ml for agar dilution tests or 1,024 μg/ml for broth dilution tests, concentrations that can be solubilized only in undiluted DMF.

While performing nitrofurantoin susceptibility tests by the agar dilution technique (1, 2), we noted a marked inhibition of growth on control plates containing 18 ml of Mueller-Hinton agar and 2 ml of DMF. A series of test plates was then prepared by adding 2.0, 1.0, 0.4, 0.2 and 0.1 ml of DMF in 20 ml of Mueller-Hinton agar. The plates were inoculated with a hand-held inoculum replicator, delivering approximately 105 to 108 colony-forming units. Tests were carried out with 36 isolates, including: 4 Staphylococcus aureus, 4 S. epidermidis, 2 Streptococcus faecalis, 2 S. faecium, 3 Proteus morganii, 1 P. rettgeri, 2 Enterobacter aerogenes, 2 E. cloacae, 4 Serratia marcescens, 4 Klebsiella pneumoniae, 4 Escherichia coli, and 4 Pseudomonas aeruginosa. The agar medium containing 2.0 ml of DMF completely inhibited all test strains for 24 h but, after 2 days of incubation, some delayed growth was seen with all four S. aureus isolates and one S. epidermidis isolate. With 1.0 ml of DMF, the isolates were all inhibited, but some delayed growth was seen with 28 of the 36 isolates. All of the organisms grew heavily on Muller-Hinton agar plates containing 0.4 ml or less of DMF.

To further study the inhibitory activity of DMF, tubes containing 20 ml of Mueller-Hinton broth and 2.0, 1.0, 0.4, 0.2, and 0.1 ml of DMF were inoculated with the "Seattle" E. coli and S. aureus (Fig. 1). Killing curves were then constructed by determining the number of viable cells recovered after 0, 1, 2, 3, 4, and 20 h at 35 C. Broth cultures containing 0.2 or 0.1 ml of DMF did not significantly affect the rate of growth of either test strain. Similar experiments were carried out comparing the rate of killing by nitrofurantoin (50 μg/ml) with and without DMF. No clear antagonism or synergism between these two drugs could be documented, but inhibitory concentrations of DMF masked the effect of the nitrofurantoin.

It was concluded that the currently recommended (1, 2) methods for performing quantitative susceptibility tests with nitrofurantoin should be modified by reducing the volume of antimicrobial stock solutions added to the agar or broth medium to avoid the addition of inhibi-
Dimethylformamide (DMF)

S. aureus

E. coli

20 ml Mueller-Hinton Broth
Plus:

---------2.0 ml DMF
1.0 ml DMF
-----0.4 ml DMF
0.2 ml DMF
0.1 ml DMF
or NO DMF

VIALBLE BACTERIA/ml (Logio)

TIME IN HOURS AT 35°C

Fig. 1. Inhibition of bacterial growth by DMF. Growth curves in Mueller-Hinton broth with 0.1 ml of DMF superimposed those obtained in control broth with no DMF.

LITERATURE CITED
