NOTES

Rapid Method of Microbial Susceptibility Testing

J. KENNETH McCLATCHY
National Jewish Hospital and Research Center, Denver, Colorado 80206

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A rapid method of microbial susceptibility testing is described. Bacterial growth is measured by determining the incorporation of 14C-tryptophan into cellular protein in the presence of antimicrobial drugs.

It is evident that the increasing availability of antimicrobial drugs and the emergence of mutants resistant to these drugs require rapid and reliable drug susceptibility data. The test usually employed measures zones of inhibition of bacterial growth around drug-impregnated paper discs placed on solid media. Less frequently, visual turbidity is measured in liquid media containing the drug to be tested. Both of these procedures require incubation periods of 12 to 24 hr for most bacteria and as long as 3 to 6 weeks for the slowly growing pathogenic mycobacteria.

This report describes a method of drug susceptibility testing which reduces the incubation period to about 3 hr for rapidly growing bacteria and to 3 days for pathogenic mycobacteria. Growth in the presence of various antimicrobial agents is determined by measuring the incorporation of a 14C-labeled amino acid into bacterial protein. The incorporation of the radioisotope serves as a sensitive indicator of growth, and an incubation period equivalent to two to three generations suffices to indicate bacterial susceptibility or resistance to the drug.

The method is carried out as follows. Several colonies of the suspected pathogen are picked from a primary isolation medium and resuspended in 2.0 ml of 7H11 broth (2) modified by the omission of the malachite green. One or two drops of this suspension are inoculated into tubes containing 5.0 ml of modified 7H11 broth containing 0.1% yeast extract and 0.1 µc of L-tryptophan-3-14C (Schwarz BioResearch, Inc., Orangeburg, N.Y.). [Although 7H11 broth was developed for the recovery of mycobacteria from pathological materials (2), it is a satisfactory growth medium for many other bacteria and fungi, especially if the malachite green is omitted and 0.1% yeast extract is added. Since the acid-hydrolyzed casein in the 7H11 medium is low in tryptophan, 14C-tryptophan is used as the radioactive amino acid.

Adequate carrier tryptophan is contained in the yeast extract.] A control culture with no antimicrobial agent is prepared simultaneously. Care is taken to ensure the addition of identical inocula to each tube. An optimal bacterial concentration in the tubes corresponds to an optical density of 0.05 to 0.1 at 600 nm (Coleman Junior II spectrophotometer, Coleman Instruments Corp., Maywood, Ill.). The tubes are incubated at 37°C on a rotating roller drum apparatus for aerobic bacteria. Anaerobes are incubated with the tubes placed in an anaerobic jar. Samples of 1 to 1.5 ml are taken at times corresponding to one, two, and three bacterial divisions. Each sample is filtered through a 0.45-µm pore size membrane filter (Millipore Corp., Bedford, Mass.) and then washed with 10 ml of cold 5% trichloroacetic acid. The filters are glued to planchets and dried, and the radioactivity is determined in a gas-flow end window counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Susceptibility to the drug tested is then evaluated by comparing the radioactivity incorporated in the presence of the drug with that in the control culture. The results are later confirmed by conventional drug susceptibility testing techniques.

The results of a radioisotopic susceptibility test on a single-colony isolate of Mycobacterium tuberculosis from an infant are shown in Fig. 1. Samples were taken after 24, 48, and 72 hr of incubation since the generation time of this organism is about 24 hr. Equivalent growth was obtained in the control culture and in the kanamycin-containing culture; the isolate was resistant to kanamycin. Streptomycin completely inhibited growth of this strain, and it was obviously streptomycin-susceptible. The culture was also susceptible to isoniazid and ethambutol. The fact that 14C-tryptophan was incorporated in the presence of isoniazid and ethambutol during the first sampling period was not unexpected since neither
of these drugs has a primary effect on protein synthesis (3, 6). A similar lag period preceding the inhibition of tryptophan incorporation in susceptible strains was obtained in other tests in the case of ethionamide, cycloserine, and p-aminosalicylic acid. Tryptophan incorporation in strains susceptible to streptomycin, kanamycin, viomycin, and capreomycin was inhibited immediately; these antibiotics are known to inhibit protein synthesis directly (5). The susceptibility patterns of mycobacterial strains were confirmed by using the routine “indirect” laboratory technique, a procedure which required a 21-day incubation period (4).

The result of a susceptibility test on a strain of Pseudomonas aeruginosa isolated from sputum is shown in Fig. 2A. It can be seen that this strain was resistant to streptomycin and susceptible to kanamycin and gentamicin. Figure 2B shows the result of a susceptibility test on a strain of Staphylococcus aureus. This organism, isolated from a wound infection, was resistant to streptomycin and penicillin G and was susceptible to methicillin and kanamycin. Since both organisms shown in Fig. 2 grow considerably faster than M. tuberculosis, samples were taken at hourly intervals for counting. The results for both strains were confirmed by the conventional antimicrobial disc technique (1).

Although results on only three different bacterial isolates are illustrated here, the method has been used successfully on a variety of other microorganisms (including Escherichia coli, Enterobacter aerogenes, Bacteroides species, and Nocardia asteroides) isolated from patients with severe infections in which speed was of the essence in establishing a proper course of chemotherapy. The technique has proved to be especially valuable for rapid susceptibility testing on isolates from positive blood cultures. The rapidity of this test also makes it particularly useful for determining microbial susceptibilities to chemotherapeutic agents such as cycloserine and cephalothin, which are relatively unstable in culture media. The procedure is technically simple and enjoys the advantage of any method in which a liquid medium is used in that one can easily use a variety of drugs, drug concentrations, and drug combinations, if desired. The primary disadvantage of this method is the requirement for isotope-counting equipment. However, gas-flow end window counters are now standard equipment in many clinical or research laboratories associated with modern hospitals.

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LITERATURE CITED


