A RAPID METHOD FOR THE DETERMINATION OF MICROBIAL SUSCEPTIBILITY USING THE FIREFLY LUCIFERASE ASSAY FOR ADENOSINE TRIPHOSPHATE (ATP)

H. Vellend, S. A. Tuttle, M. Barza, and L. Weinstein
New England Medical Center Hospital
Boston, Massachusetts

Grace Lee Picciolo and Emmett W. Chappelle
Goddard Space Flight Center
Greenbelt, Maryland

ABSTRACT

The luciferase assay for adenosine triphosphate (ATP) was optimized for pure bacteria in broth in order to evaluate if changes in bacterial ATP content could be used as a rapid measure of antibiotic effect on microorganisms.

Broth cultures of log phase bacteria (10^6 colony-forming units/ml) were incubated at 310 K (37°C) for 2.5 hours at antimicrobial concentrations which resulted in the best discrimination between "sensitive" and "resistant" strains. ATP assays were performed on the control broth culture at the onset of incubation (A_0) and again after 2.5 hours on both the antibiotic-containing broth culture (B_t) and the antibiotic free growth control (A_t). The drug effect on bacterial ATP content was quantitated using the following formula:

$$\text{ATP Index} = \frac{\log B_t - \log A_0}{\log A_t - \log A_0}$$

Empirical observations from a large number of microbial susceptibility tests performed by this method suggested that an index of $> +0.25$ implied resistance and $\leqslant +0.25$, sensitivity.

Eighty-seven strains of 11 bacterial species were studied for their susceptibility to 12 commonly used antimicrobial agents: ampicillin, Penicillin G, nafcillin, carbenicillin, cephalothin, tetracycline, erythromycin, clindamycin, gentamicin, nitrofurantoin, colistin, and chloramphenicol. An overall comparison of the results obtained by the ATP index and agar diffusion sensitivity testing demonstrated a 90 percent agreement. Of the 10 percent

instances of disagreement, most (three-fourths) were major, i.e., false-resistance or false-sensitivity. One-quarter of the disagreements were minor, i.e., intermediate by agar diffusion and either sensitive or resistant by the ATP index. The principal cause for major disagreement between the ATP index and agar diffusion appeared to be related to the mode of action of the antimicrobial agent. The reproducibility of the method was entirely satisfactory (94 percent).

The major advantage of the ATP system over existing methods of rapid microbial susceptibility testing is that the assay can be made specific for bacterial ATP. This unique feature may allow this technique to be applied directly to organisms in urine or other biological fluids without prior bacterial isolation or subculture. Studies to this effect are in progress.