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Laboratory Control of Chemotherapy

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New Antibacterial Antibiotics

Clinical Evaluation of Antibacterial Chemotherapeutics in Internal Medicine

Clinical Evaluation of Antibacterial Chemotherapeutics in Surgery

Clinical Evaluation of Antibacterial Chemotherapeutics in Urology

Clinical Evaluation of Antibacterial Chemotherapeutics in Dermatovenerology

G. Anderson[1]

The Role of the Laboratory in Antimicrobial Therapy

In this era of specific antimicrobial chemotherapy a major function of the diagnostic microbiology laboratory is not exactly to act as a controlling service in the treatment of microbial disease but rather to serve, as it does, as an important guide to chemotherapy through the intelligent application and interpretation of *in vitro* sensitivity tests in the selection of antimicrobial agents.

Today the minimal inhibitory concentration (MIC) of an antimicrobial agent for a microorganism has precedence over the specific identification of the etiologic agent. This is possibly as it should be, for in certain respects it makes little difference what the specific organism is, providing some agent can be found that will aid in bringing about its eradication. It is not uncommon, therefore, for the physician in considering the possible microbial etiology, in support of his clinical diagnosis, to think of a microorganism in a very general way and excluding viral, rickettsial, fungal, parasitic, or spirochaetal etiology, reflect on the possibility of an etiology that is Gram-positive or negative by nature, and since the microbial agents available today cover in their collective spectrum most, if not all, members within that broad microbial menage that can be separated by the Gram stains, the generalization is acceptable. It is not and should not be used, of course, solely in lieu of definitive laboratory confirmation, identification and, in turn, specific sensitivity information. *Jawetz*[1], writing in a light but serious vein, states: "And yet as a first step in rational selection of drugs we must accept the fact that antibiotics are not tonics. Their therapeutic activity depends upon their ability to inhibit or kill microorganisms. Before administering such a drug the physician must therefore convince himself that the patient suffers from a microbial infection." I would add to that that the organism is sensitive to the agent and, in turn, that the patient is not subject to that physiologic anomaly of being sensitive. *Stewarl*[2] stressed the situation this way: "The intelligent use of antibiotics requires some attention to biology as well as to therapeutic impulses."

The laboratory is considered to be the place where theory is tested, proven to be correct or incorrect, and fact is established. However, methodology with respect to *in vitro* antimicrobial sensitivity testing within the multitude of world-wide laboratories is variable and contains many variables, and there is no *in vivo* testing method short of administration of the chemotherapeutic agent to the patient which, in turn, is guided by some form of an *in vitro* test.

By and large the test most universally used is the simple agar disc diffusion test which in essence had its inception in that momentous observation of *Dr. Fleming*[3]. However, a test upon the results of which such great responsibility lies, a test with such great potential, is a test which within itself needs guidance. In other words, a reference. I made reference to this need for standardization 12 years ago[4]. I know I was not alone in that desire. The World Health Organization, through Report 210[5], recognized this need for standardization of antimicrobial sensitivity testing. Today there is still no international reference standard for antimicrobial sensitivity testing by any method that may be used, be it agar diffusion, agar dilution or test tube dilution. Some direct but not completely acceptable progress has been made, parti-

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cularly with respect to the agar disc diffusion method.

The "International Collaborative Study of Antibiotic Sensitivity Testing" (ICS) has been completed and a report has been published[6]. Progress in that study initiated by *Dr. Ericsson* of the Karolinska sjukhuset, Stockholm, Sweden, sponsored by the World Health Organization and in which scientists in 16 laboratories participated, took time, if for no other reason than the complexities inherent in its scope. Practically all phases of the agar disc diffusion method were scrutinized and comparative data with broth dilution and agar dilution methods collated to provide a basis for a reference standard method in each category. It was not the purpose of the ICS, as has been previously stated[7], to devise a method for sensitivity testing above all others, but it was the purpose of the ICS to establish acceptable guidelines and make recommendations for reference standards that could be universally applied to antibiotic sensitivity testing. Time permits only a restatement of some of the recommendations pertinent to the ICS and I quote: "The effective application of reference procedures, and improvement in the general quality of the routine performance of *in vitro* sensitivity tests will be dependent in part on the availability of reference laboratories of expert advice and of methods for performance evaluation. Much of this service can best be provided by experienced national laboratories because of differences in technical conventions and of antibiotic usage."

Through the establishment and use of expert or reference laboratories and the combined and cooperative use of experts in microbiology, government agencies controlling chemotherapeutics, pharmaceutical industries and wellinformed experts in clinical infectious diseases, the quality of sensitivity testing in the routine diagnostic laboratory, wherein the greatest problem lies, could be and would be materially elevated. A working basis to bring about this quality control in sensitivity testing would be the reference methods, reference cultures, a reference medium and control of disc performance and potency. The latter is most significant to the success of the program, for which purpose the ICS recommends the performance standard test of the U. S. Food and Drug Administration. Without control of disc content in the diffusion test, any degree of proper test interpretation, which is so vital, is impossible. I have always believed the antibiotic content of the disc should be optimal to detect organisms that would be considered sensitive, but not of that order to bring into the sensitive range the culture which, by its heterogeneity, should be relegated to that equivocal interpretation of being less than sensitive, but not quite resistant. Apropos to the equivocal interpretive zone referred to by some as "Moderately Resistant", that interpretation should be deleted and the sensitivity of any organism falling into that category should be more specifically determined by the agar dilution or test tube dilution method to afford the physician more specific information, however well he might be able to interpret the significance of the quantitative report versus the qualitative. The determination of sensitivity by the new automated procedures is quantitative which, of course, has merit. However, where the physician has been accustomed to accept the simple term "Sensitive" as probably synonymous with successful therapy, it will take time to orient him to the significance of sensitive to 1.0 μg vs. 100 μg. We all recognize, of course, the difference in the significance of such a quantitative determination relative to a body site where antimicrobial agents may be concentrated. It would seem well, then, to place the laboratory report in the proper perspective relative to a quantitative report to place some credence in the recommendation of the ICS and think of adopting a categorized report with designations such as Group I, II, III and IV, as it will relate sensitivity more specifically to proper use of the antimicrobial agent. Interpretation and reporting results using the Group scheme was presented early in the antibiotic era by *Ericsson* and has been in use in Sweden[8]. Its use may require closer cooperation between laboratory and physician, but that aspect of sensitivity testing and interpretation is definitely necessary.

In conclusion, time has permitted only some generalizations on the role of the laboratory as a guide to antimicrobial

chemotherapy. The sensitivity test is that guide. There are many methods with which this can be done. In essence each nation has its own methods which, though similar in most respects, are each intrinsically different, each serving the same purpose to show *in vitro* that the etiologic agent of an infectious disease will be inhibited by a chemotherapeutic agent.

The most widely used method, because of its simplicity and economy, is the agar disc diffusion method. However, there are variables in that method that could account for marked variation in results from one laboratory to another. There is need, then, for standards of reference not only on a national, but on an international basis. Guidelines for standards of reference have been set forth in the Report of an International Collaborative Study of Antibiotic Sensitivity Testing. To expect all to agree would be asking the impossible. Areas such as a medium, disc content, reference cultures and interpretation need further consideration and agreement. There is need for reference laboratories and experts to act collectively to advise, accept and recommend not only the best methods for testing, but also how the results of those tests may be applied to the greatest advantage for the patient. Experience dictates that what has been done in the laboratory as a guide to chemotherapy deserves meritorious recognition. However, there is always room for improvement.

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Application of Sensitivity Discs to Some Clinical Laboratory Tests for Chemotherapy

The sensitivity disc method is now widely used in many laboratories because of its simplicity and availability on multiple drugs. We have studied the disc assay technique as to its usefulness in detecting various attributes of infecting microorganisms and of antimicrobial agents as well as a sensitivity test. The studies have demonstrated the practical usefulness of the technique as a simple test for bacterial development of drug-resistance and for bacterial inactivation of antimicrobial agents[1,2,3]. In this paper we present the results of some subsequent studies.

1. Procedural simplification with respect to reading the results of the sensitivity test

While application of the single-disc method in the sensitivity test has the advantage of procedural simplicity, it has the disadvantage that reading the results is inevitably complicated. Using diagrams with translation the diameter of inhibition zone into the degree of sensitivity, we have elaborated a very simple mode for reading the results (Fig. 1). Taking the measures of inhibition zones by means of slide caliper in the usual manner, and, without reading it, we transfer the caliper mark on the diameter directly (arrow) and then record the corresponding degree of sensitivity in order to use the forms as assay data sheets, as they are. This not only permits instant reading of sensitivity (e. g. —, + .., R. S. ...), but also has the advantage of immediatelly obtaining the approximate MIC values from a previously prepared calibration chart as well.

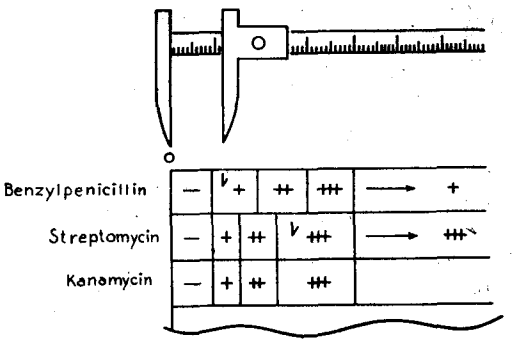

Fig. 1. Translation of the diameter of inhibition zone into the degree of sensitivity.

2. Simple test for the detection of drug-induced resistance of Staphylococci

Recently, it has been considered clinically important to detect and foresee the possibility of drug-induced resistance before choosing a proper agent or discontinuing it and/or replacing it by an other drug, in the case of treatment with macrolide antibiotics in staphylococcal infections. A simple technique enabled us to detect the phenomenon simultaneously with a number of chemotherapeutic agents.

In our test, a disc of the resistance-inducing drug was placed onto the center of a seeded agar plate. After incubation for 2—3 hours, discs of drugs to be tested were arranged around the central discs in adequate distances, then the plate was further incubated to be read for the formation of inhibition zones. Because of the reduction of the inhibition zone produced by Staphylococcus that had aquired resistance to the drug in consequence of contact with the resistance-inducing drug in the centrally placed disc, it was possible to detect readily the development of induced

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resistance (Fig. 2). The results with Staphylococci isolated from clinical material, as shown in Table 1, indicated the practical usefulness of this simple technique as screening test.

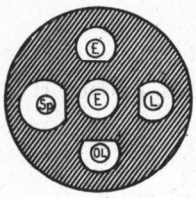

Fig. 2. Simplified test for the detection of inducible drug-resistance.
E = erythromycin 50 μg, Sp = spiramycin 30 μg, L = kitasamycin 30 μg, OL = oleandomycin 30 μg.

3. Simple method for the detection of the transferable drug resistance factor (R) with some strains of enteric bacteria

In the treatment and epidemiology of infections caused by enteric bacteria, it has become important to clarify the state of the transferable resistance factor (R). Detection of the R-factor is usually accomplished by the use of mixed bacterial culture, which is a rather too cumbersome procedure to be adopted for routine labora-

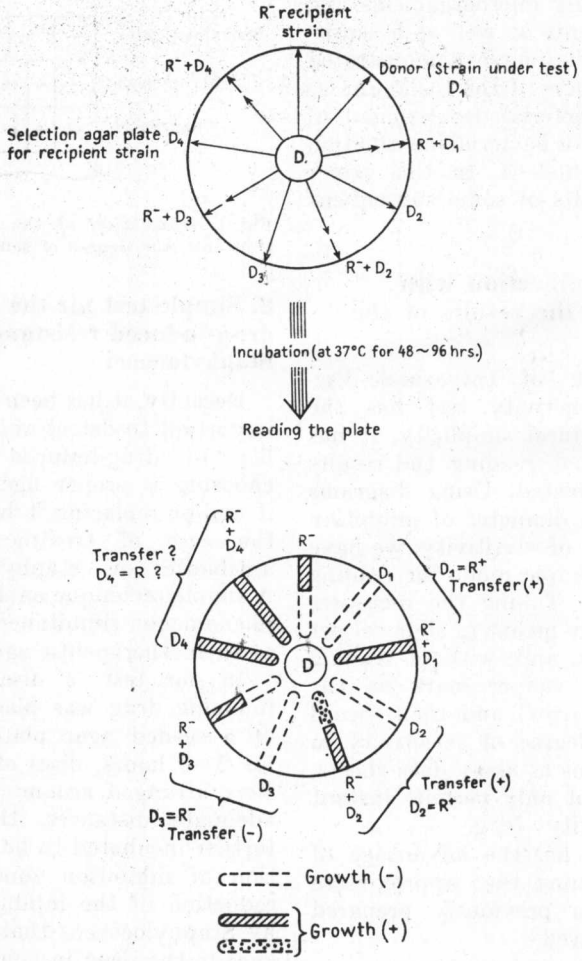

Fig. 3. Streak-seeding method (for detection of transferable drug resistance factor). A growth of the recipient strain (R^- strain) or that of the donor strain (under test), or both, are seeded by radially streaking on the plate with a sensitivity disc at the center.