THE ADOPTION OF A STANDARDIZED ANTIBIOTIC SENSITIVITY TEST IN THE PHILIPPINES

Dr. Thelma Tupasi-Ramos
College of Medicine

I. INTRODUCTION

With the development and the introduction of a great variety of antibiotic and chemotherapeutic agents, the outlook in the treatment of infections has improved significantly. Unfortunately, however, these agents are not necessarily innocuous to human tissues, so that their use in some instances is associated with some potential hazards including tissue toxicity, hypersensitivity reaction, emergence of bacterial antimicrobial resistance and the development of clinical superinfection. In view of these hazards, therefore, the administration of an antibiotic must be initiated only when there are definite objective evidences of an infection from clinical and laboratory parameters. Furthermore, the choice of antibiotic must be based on objective results of the antibiotic sensitivity test done on the isolated etiologic agent.

There are several methods of antibiotic sensitivity testing, but the method that finds greatest applicability is the disc diffusion test. This consists of applying an antibiotic containing disc or an agar medium which had been previously inoculated with the test organism. As the name implies, antibiotic diffuses from the antibiotic disc radially out into the agar so that immediately near the periphery of the disc, the concentration of the antibiotic would be highest and the concentration would decrease exponentially the further the distance is from the disc. The results are read after 18 months incubation as zones of inhibition around the antibiotic disc. Organisms that are susceptible to the antibiotic would be inhibited by low concentrations of antibiotics and would thus have large zones of inhibition. In contrast, a resistant organism would require high concentrations of the antibiotic to inhibit its growth and in some instances may not be in-

hibited at all. Thus, very narrow zones or no zones at all would be observed around the antibiotic to which the organism is resistant[2].

In addition to the susceptibility of the organism, several other factors which are basically technical have also been found to affect the size of the zone of inhibition. These factors include the size of the inoculum, various factors in the culture media used and the antibiotic disc potency. The size of the zone of inhibition is inversely related to the inoculum size. There are four major factors in the culture media that may affect in-vitro susceptibility test, namely, pH of the culture medium, the stability of the antibiotic in the culture medium, growth supporting capacity of the culture medium, and the antibiotic binding factors in the culture medium[3, 4, 5]. As far as antibiotic disc potency is concerned, there would be a direct relationship with the size of the zone of inhibition in that a high potency antibiotic disc would understandably have bigger zones than an antibiotic disc that has a lower potency.

Because of the possible variability in zone sizes that may result from variability in the technique used, the need to standardize the procedure has been recognized. Moves for standardization of the procedure started way back 20 years ago in the United States, but has only gained momentum in the past 6-7 years when the United States Food and Drug Administration took an active part in organizing a working committee to study and make recommendation for a standardized procedure and finally in issuing directives making the performance of antibiotic sensitivity test according to the standardized procedure compulsory[6]. In the international level, the movement for standardization likewise has become more intense and in 1971 the recommendation of the WHO collaborative study group were formulated[7].

The standardized antibiotic sensitivity test that is now accepted worldwide is the disc diffusion test carried out according to the technique of Kirby-Bauer[8]. These are two pioneers from the University of Washington in Seattle who as early as 20 years ago already realized the need for stan-

dardization and hence, they developed a single high potency disc sensitivity test method. The term "single high potency" is used here to differentiate it from the old system when three discs of an antibiotic of varying potencies were used. The test according to the K-B technique calls for a constant method of performing the test, with the aim of approximating in-vivo conditions as much as possible. This included:

1. the use of inoculum standardized by gross comprises with a MacFarland Turbidity Standard 0.5 which is approximately equal to that turbidity due to 10^6 Enterobacteriacose/ml.;

2. the use of Muller Hinton medium which is free of any antibiotic antagonist, and adjusted to the physiology pH range of 7.4. Other accepted substitutes include axoid, DST and SAF agar; and

3. the use of one antibiotic of high potency to represent an antibiotic group (2).

As a result of standardizing the procedure as such, it has also been possible to arrive at a more objective interpretation of the result which is quantitative and more clinically applicable.

Realizing therefore the vital importance of the antibiotic sensitivity test for the clinician who is confronted with the problem of treating a patient with infection and realizing further the need for adopting a standardized method in order to arrive at a more objective and clinically more meaningful results, this project was undertaken with the following objectives:

1. to introduce the concept of a standardized antibiotic sensitivity test and to encourage its adoption as a routine laboratory procedure;
2. to train the medical microbiologist the technique of performing the standardized antibiotic sensitivity test;

3. to initiate a surveillance program of antibiotic sensitivity patterns of common bacterial pathogens;
4. to establish a surveillance program of the antimicrobial susceptibility among isolated bacterial pathogens in different regions in the country. Data gathered from this surveillance program are intended guides for clinicians in the initial therapy of infection encountered in their locality pending availability of antibiotic sensitivity test on the isolated pathogens.

The first two objectives have been realized during the first two years of the project and the observations made are herein reported.

II. MATERIALS AND METHODS

Since February, 1974 we have conducted teaching seminars participated in by clinical pathologists and medical technicians from all over the country. A pre-seminar evaluation to determine current status of antibiotic sensitivity test is done by letting the participants perform the test in a manner that is routinely done in their laboratories.

The seminar is a four-day course consisting of didactic lectures on the mechanics and basic principles of the standardized antibiotic sensitivity test and actual bench work by the participants under the guidance of our research assistants. The importance of standardizing the inoculum, media, and antibiotic disc potency is emphasized and the participants are actually made to do the procedures by themselves repeatedly so that at the end of the four days, they have gained enough proficiency to undertake the procedure in their own laboratories.

Follow-up visits by a research assistant to the laboratories of the participants are periodically undertaken to encourage the participant in the adoption of the standardized sensitivity test and to identify problems encountered so that appropriate steps may be taken to solve these problems.

III. RESULTS AND DISCUSSION

Seminar participation. Table I shows the number of participants among the eighty-two diagnostic laboratories currently undertaking bacteriology work in the Philippines. Sixty-eight of these laboratories plus sixteen other laboratories currently not doing bacteriology work were represented by one hundred thirteen clinical pathologists and medical technicians during the thirteen seminars conducted. The response among the participants was enthusiastic. During the first few seminars the transportation and living expenses of the participants were fully subsidized by the project; later, however, when the subsidy had been withdrawn, the participating laboratories still sent and supported their own representative.

Pre-seminar evaluation. Results of the pre-seminar evaluation are presented in Table II. The variability of antibiotic sensitivity test that is routinely performed by diagnostic laboratories is evident from this table. Standardization of the inoculum was done by eleven of the one hundred thirteen participants. There was no uniformity to test media used and many laboratories performed antibiotic sensitivity test using several types of media, obviously depending upon what was available to them in the laboratory. The antibiotic potency of the discs used was likewise not uniform, the majority of the responders used multiple potency of antibiotics.

Standardization of the antibiotic sensitivity test would allow for a more objective interpretation of the results which is quantitative and more clinically applicable. This is done by correlating the measured zone of inhibition to the minimum inhibitory concentration of the antibiotic by means of a regression analysis, bearing in mind the expected blood vessels attainable with the usual therapeutic dose[9]. The interpretation of results as routinely done, however, was arbitrary and variable, with the majority of respondents interpreting

results subjectively without measuring the zone sizes.

The standardized antibiotic sensitivity test may be more clinically applicable by limiting the antibiotic used in testing a particular pathogen only on those that have been found to be clinically effective[10]. Inclusion of an antimicrobial agent in the test is taken as an implied endorsement of that particular antimicrobial agent in the treatment of the infection; hence to avoid misleading the clinicians, the antibiotics tested should only include those that are clinically useful. Again, observations from our pre-seminar evaluation show that the choice of antibiotics used in testing the organism was very variable. Table III shows the antibiotics used by the participants against Staphylococcus aureaus. The antibiotics recommended for testing this particular pathogen are underscored and include Cephalothin, Penicillin G, and Isoxazolyl Penicillin, namely, oxacillin and dicloxacillin. Tetracycline and Ampicillin were more frequently used by the participants compared to the recommended antibiotics. In addition, other drugs that are probably not clinically useful, such as Nitrofurantoin and Nalidixic acid were also included.

Effect of variability of test conditions on routine antibiotics sensitivity test. As a result of variability in test conditions, discrepancies in the results obtained by the participants were noted when compared with categorization based on results of disc diffusion test correlated with antibiotic dilution team done in the research laboratory.

Table IV shows the categorization of the organism based on the sensitivity test done by the participants compared to the results of sensitivity test in correlation with the minimum inhibitory concentration obtained by antibiotic dilution test. The Staphylococcus aureaus used was sensitive to Penicillin G, Cephalosperine, and Oxacillin. Twelve, 8 and 28 percent of the participants categorized other organisms as resistant to Penicillin G, Cephalospherine, and Oxacillin, respectively. The Klobsiolla specie tested was sensitive by correlative studies with disc diffusion and antibiotic dilution test to

Cephalosperine Colistin; 67 and 9 per cent of the participants categorized it as resistant to the respective antibiotics. Discrepancies were also noted with the other antibiotics; 40, 93, 62, 40 and 87 per cent of the participants categorized the organism as sensitive to Ampicillin, Gentamicin, carbenicillin tetracycline and co-trimoxazole, respectively, in contrast to demonstrated in-vitro resistance of the organisms to these antibiotics.

Implementation of Standardized Antibiotic Sensitivity Test by Participating Laboratories. Table V shows the number of the participating laboratories which have adopted the standardized sensitivity test after the seminar attendance. While the results are gratifying in the prominent laboratories, only six of thirty-eight laboratories in the greater Manila area have adopted the standardized procedure.

IV. CONCLUSION

The need to standardize antibiotic sensitivity test is underscored by the prevailing variability in techniques and interpretation routinely used by diagnostic laboratories locally.

The apparent unenthusiastic adoption of the standardized sensitivity test by diagnostic laboratories is due largely to the lack of authority on our part to compel them to undertake the standardized procedure as their routine method and also due to invariability of the materials needed.

From the American experience, it has been clearly demonstrated that a directive from a regulating agency like the Food and Drug Administration of the Department of Health and Welfare is a very effective way of enforcing this standardized procedure as a compulsory method[6].

Secondly, a government-subsidized program of importation and/or local production of these necessary materials would be the solution to the current invariability and "black marketing" of the little supply that is available.

TABLE I: Séminar Participation

AREA	PARTICIPATING LABORATORIES	TOTAL NUMBER OF LABORATORIES
LUZON		
Greater Manila	38	46
Baguio City	1	2
San Fernando, L.U.	2	2
Others	5	5
VISAYAS		
Cebu City	8	11
Bacolod	6	7
Dumaguete	2	2
Tacloban	1	1
Ormoc	1	1
MINDANAO		
Cagayan de Oro	1	1
Cotabato	2	2
Davao	1	2
TOTAL	68	82

TABLE II: Pre-seminar Evaluation
Responders: 113

INOCULUM	
STANDARDIZED	11
NOT STANDARDIZED	102
MEDIA	
MUELLER HINTON	26
DST	18
Blood	72
EMB	28
Nutrient	27
Chocolate	18
Mac Conkey	12
Others	2
ANTIBIOTIC DISC	
High Potency	37
Low Potency	9
Multiple Potency	61
INTERPRETATION	
ZONES MEASURED	51
ZONES NOT MEASURED	62

TABLE III: Variability in Antibiotics Tested
Organism: Staphylococcus Aureus

Tetracycline	— 86%	Kanamycin	— 41%
Ampicillin	— 80%	Colistin	— 36%
Cephalothin	— 72%	Mitrofurantoin	— 18%
Penicillin G	— 65%	Erythromycin	— 12%
Oxacillin	— 60%	Chloramphenicol	— 8%
Gentamicin	— 58%	Dicloxacillin	— 2%
Cerbenicillin	— 48%	Malidixic acid	— 1%
Co-trimoxazole	— 47%	Sulbenicillin	— 1%

TABLE IV: Discrepancies in Results of Antibiotic Sensitivity Test

	ORGANISM:	Per Cent of Participants Categorizing the Organism as Sensitive	Resistant
1.	Staphylococcus aureus		
	Penicillin G Sensitive	88%	12%
	Cephalosporin Sensitive	92%	8%
	Oxacillin Sensitive	72%	28%
2.	Klebseilla spp.		
	Cephalosporin Sensitive	33%	67%
	Ampicillin Resistant	40%	60%
	Gentamicin Resistant	93%	7%
	Colistin Sensitive	91%	9%
	Carbenicillin Resistant	62%	38%
	Tetracycline Resistant	40%	60%
	Co-trimoxazole Resistant	87%	13%

TABLE V: Implementation of Antibiotic Sensitivity Test

Area	Laboratories with Procedure Standardized	Participating Laboratories
LUZON		
Greater Manila	6	38
Baguio	1	1
San Fernando, L.U.	2	2
Others	5	5
VISAYAS		
Cebu City	6	8
Bacolod	6	6
Dumaguete	2	2
Tacloban	1	1
TOTAL	29	63

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